EFECTOS DE LA DIÁLISIS Y DEL ESTADO URÉMICO SOBRE LA MODULACIÓN DEL APETITO Y EL SINDROME MIA (MALNUTRICIÓN, INFLAMACIÓN Y ATEROESCLEROSIS) EN PACIENTES EN DIÁLISIS.

Memoria para optar al título de Doctor en Medicina presentada por:

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A mis padres

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1.- Abreviaturas

AgRP: proteina relacionada a agouti

BCCA: amino ácidos ramificados de cadena corta

BMI: índice de masa corporal

CCK: colecistoquinina

DP: diálisis peritoneal

FRR: función renal residual.

GH: hormona del crecimiento

GIP: péptido inhibidor gástrico o péptido insulina trópico dependiente de glucosa

HD: hemodiálisis

IL-1: interleuquina 1

IL-6: interleuquina 6

MC4-R: receptor 4 de la melacortina

MIA: síndrome malnutrición, inflamación y ateroesclerosis.

MSH: hormona estimulante de la mealnocortina

NO: oxido nítrico

NPY: neuropéptido Y

SNC: sistema nervioso central

TNF-α: factor de necrosis tumoral

2.- Introducción

La anorexia es el primer obstáculo que puede tener el paciente urémico para asegurar una ingesta dietética adecuada. Aunque el síntoma se asocia a la misma uremia, especialmente cuando ésta es extrema, puede ser muy frecuente y sobre todo persistente. Los reconocidos efectos nocivos de la malnutrición justifican toda profundización en este tema.

2.1.- Fisiología normal de la conducta alimentaría.

El ciclo hambre-saciedad es controlado por un sistema complejo y heterogéneo de autorregulación que incluye el tracto gastrointestinal, el hígado, los nutrientes circulantes, los depósitos de grasa, el metabolismo celular y el sistema de transmisión del mensaje (sistema nervioso periférico) al sistema nervioso central (SNC) (1-4). En el SNC existe un centro llamado del hambre que está localizado en el hipotálamo lateral y otro centro, el de la saciedad, situado en el hipotálamo ventromedial. Otras áreas del cerebro como el núcleo paraventricular, las áreas tegmentales ventrales, la amígdala, el globus pallidus y el área postrema están también implicadas en el control del apetito (3).

Fases que regulan el ciclo hambre-saciedad.

A.- La fase gástrica empieza cuando el alimento llega al estómago produciendo distensión de sus paredes, con la consiguiente sensación de plenitud. Posteriormente, los nutrientes inducen liberación de péptidos GI con efecto saciante: Colecistoquinina (CCK), gastrina, somatostatina y bombesina. La CCK es uno de los pépticos más importantes en la regulación del apetito, atribuyéndosele un doble efecto saciante, uno central y otro periférico (control periférico del apetito) (5). En esta fase, el tipo de alimento ingerido tiene importancia, ya que por ejemplo, la ingesta de hidratos de carbono produce

saciedad para los mismos pero no para las proteínas (6). El mensaje es finalmente llevado al SNC por el nervio vago, concluyendo la fase gástrica.

B.- La siguiente es la fase post-absortiva. Para explicar esta fase existen varias teorías que asocian el efecto de los nutrientes y sus formas de reserva corporal con la regulación del apetito (teorías lipostática, glucostática y aminostática) (7). El descubrimiento de la leptina confirma estas ideas. Esta hormona segregada por los adipocitos, cuyo nivel plasmático refleja las reservas grasas corporales, inhibe el apetito mediante lo que podría considerarse un feed-back negativo de mantenimiento del peso corporal (8). Recientemente se ha postulado que el mensaje de la depleción nutritiva viene dado por la tasa de utilización-producción celular de energía a partir de los alimentos (9). Smith GB et al (10), plantearon que el control alimenticio podría venir de signos endógenos producidos en el intestino como consecuencia de la absorción alimenticia. En cualquier caso, la presencia de cualquier tipo de metabolito de los alimentos en la sangre (aminoácidos, glucosa, ácidos grasos, lipoproteínas) son capaces de inhibir el apetito (11).

C.- La fase hepática. Varios estudios implican receptores vagales y hepáticos en el control del ciclo hambre-saciedad. La concentración de ATP (adenil-trifosfato) en los hepatocitos es un fuerte estímulo para regular la sensación de hambre (12, 13).

D.- La fase central de regulación del apetito incluye neuropeptidos (Neuropéptido Y o NPY y CCK) y neurotransmisores (serotonina), capaces de inhibir o estimular el apetito a través de mensajes que son captados por el SNP (7). La concentración intracerebral o sanguínea de algunos aminoácidos precursores de la síntesis de neurotransmisores, juegan un papel importante en

esta fase de control del apetito. Por ejemplo el triptofano es el sustrato para la síntesis de serotonina. La serotonina es el principal regulador central del apetito, de tal manera que niveles elevados de esta inducen saciedad central (14, 15).

2.2.- Medición del impulso a comer, escala análoga visual (EAV) y otras escalas.

Para definir los trastornos en el hábito alimentario es necesario evaluar aspectos biológicos, sociales y culturales. Existen algunas encuestas que evalúan el impulso a comer. Algunas de estas han sido utilizadas en pacientes en diálisis, tal es el caso de la escala analógica visual (EAV), que fue utilizada por primera vez en pacientes en diálisis peritoneal y transplantados por la Dra. Hylander B, et al (16). Otra encuesta utilizada en la misma población es la utilizada por Wright MJ, et al (17) en pacientes en hemodiálisis. En este caso nosotros seleccionamos la EAV con el fin de validarla para nuestra población y para patologías concretas como la anorexia o bulimia-obesidad en el estado uremico. La EAV consiste en 5 preguntas que deben responderse antes y después de comer. Las preguntas son: deseo de comer, hambre, sensación de vacío y plenitud, cantidad de comida que comería y placer de comer en ese momento.

Otro aspecto poco estudiado en los pacientes en diálisis son las preferencias y rechazos alimenticios. Tradicionalmente se sabe que los pacientes uremicos muestran una tendencia universal a consumir carbohidratos y rechazo a carnes rojas, comparado con la población normal. Estos aspectos fueron evaluados a través de la encuesta de preferencias alimenticias utilizada por Dobell E, et al (18). En contraposición, la ingesta de carnes blancas fue menos problemática. Los pacientes urémicos también muestran una marcada

atracción por los cítricos y sabores fuertes en general. Las preferencias y rechazos alimenticios han sido relacionados a la predominancia de algunos pépticos moduladores del apetito. Niveles elevados de CCK (un poderoso anorexigeno) son asociados con saciedad temprana a carbohidratos, y NPY (un orexigeno) con repetidas ingesta alimenticia. En la población en diálisis, la asociación entre estos péptidos y los trastornos en la preferencia o rechazo a alimentos tampoco ha sido estudiada.

2.3.- Hipótesis propuestas para la anorexia y malnutrición en pacientes en diálisis.

Hablar de los trastornos en el apetito en la uremia resulta difícil por la ausencia de estudios específicos y la dificultad intrínseca para medir un síntoma tan subjetivo, multifactorial y contaminado por la propia enfermedad (16). Sin embargo, el área es de enorme interés por la elevada morbi-mortalidad asociada a malnutrición (19, 20).

Un factor importante propuesto para la génesis de la anorexia del paciente en diálisis es la dosis de diálisis (21, 22). Existe la idea de que la acumulación de una o varias sustancias tóxicas de peso molecular intermedio, sean las responsables de la anorexia urémica (hipótesis de las medianas moléculas) (23). Bergström et al (24), aislaron una mediana molécula del ultrafiltrado de pacientes en hemodiálisis (HD) capaz de suprimir el apetito. Sin embargo, estas sustancias no han sido plenamente identificadas, estudiadas y reconocidas por otros grupos a pesar del paso de mucho tiempo. Además es demasiado frecuente la existencia de pacientes con buenos índices de diálisis que presentan anorexia y malnutrición. La hipótesis de los "picos" de toxicidad sugiere que los pacientes en HD están expuestos a picos de urea y medianas moléculas durante los

periodos interdiálisis, que les suprimen el apetito (25). Sin embargo, no explica la anorexia de los pacientes en diálisis peritoneal (DP), en los que dichos picos no se producen. La ausencia de función renal residual es frecuente en los pacientes con anorexia en diálisis (26-28), apoyando la idea que la acumulación de productos tóxicos no aclarados adecuadamente por las distintas formas de diálisis, podrían ser la causa de la ausencia de apetito. En los pacientes en DP el suministro constante de glucosa por el peritoneo supone un freno al apetito, ya que los niveles séricos de glucosa son el primer inhibidor del apeptito conocido (2, 3). Nosotros hemos calculado que el suministro de glucosa peritoneal en nuestros pacientes puede rondar entre 400 y 1200 kcal/día. Lo que condiciona además hiperinsulinismo, dislipemias y major riego cardiovascular (17, 18, 28).

Otra hipótesis que explica la toxicidad de la uremia y de forma particular la génesis de la malnutrición y la falta de apetito, es el síndrome MIA (malnutrición, inflamación y ateroescleroisis) (29). El MIA se basa en la retención por falta de excreción renal o hiperproducción de moléculas pro-inflamatorias con acción caquetizante como el factor de necrosis tumoral-alfa (TNF-α) y la interleuquina-6 (IL-6). La hiperproducción se realizaría por órganos inflamados, enfermos, el peritoneo o por el estimulo constante que produce el contacto de polimorfonucleares de la sangre, con las membranas de hemodiálisis (30). Finalmente, este exceso de moléculas pro-inflamatorias produciría inhibición del apetito actuando directamente sobre el hipotálamo, inhibición del la motilidad del tracto gastro-intestinal (TGI), inhibición de la síntesis hepática de albúmina y caquexia muscular actuando en la vía de las ubiquin-proteasas (31). Sin embargo, podemos mencionar al menos dos críticas que cuestionan el MIA:

1.- En animales de experimentación la administración de TNF-α recombínate produce tolerancia y sus efectos caquetizantes desaparecen entre 1 y 12 días después de su administración (32).

2.- De la misma manera en que se retienen molecular pro-inflamatorias, se retienen también moléculas anti-inflamatorias. ¿Cual sería la resultante de este desequilibrio?. Seria necesario analizar estas últimas moléculas y definir su papel en el MIA (33).

Ante esta situación en 1995, empezamos a estudiar los aspectos biológicos del ciclo hambre-saciedad en pacientes en diálisis. Fue así como planteamos la hipótesis del desorden en el triptofano-serotonina para explicar la falta de apetito en los pacientes en diálisis (34). Esta hipótesis plantea que existe un exceso de triptofano libre que atraviesa la barrera hemato-encefálica. Este triptofano que es el sustrato para la síntesis de serotonina, induciéndose así anorexia. El exceso de triptofano libre en el líquido cefalorraquídeo se produciría por una disminución en los amino ácidos ramificados de cadena corta (BCCA), que a su vez están disminuidos por condiciones propias del estado uremico, como niveles elevados de citoquinas pro-inflamatorias, peptidos gastrointestinales, CCK, ghrelina, leptina, insulina, catecolaminas, acidosis, sobrehidratación y niveles bajos de NO (anexos I, fig.1).

La última hipótesis ha sido propuesta en 2005 por Cheung W et al (35), quienes encontraron un desorden en el receptor del gen de la melanocortina-4 (MC4-R). Este receptor se encuentra justo al final de la cascada del ciclo hambre-saciedad y su sobre-expresión disminuye el apetito. En los pacientes uremicos hay un trastorno en la habilidad para regular dicho receptor.

3.- Hipótesis y Objetivos

La observación de pacientes en diálisis que sufrían una dramática falta de apetito a pesar de mostrar criterios de diálisis adecuada junto con la ausencia de otros factores que puedieran explicarla, nos impulso a formular la hipótesis de que otros elementos biológicos y metabólicos tuvieran una mayor relevancia en la regulación del apetito.

De esta manera nuestros objetivos fueron:

1.- Definir y validar una unidad de medida del impulso a comer para conseguir de forma clara un diagnostica de anorexia y/o bulimia-obesidad

2.- Caracterizar y establecer factores de riesgo para sufrir falta de apetito en pacientes en diálisis

3.- Identificar el papel de sustancias biológicamente activas retenidas o hiperproducidas (citoquinas, peptidos…etc) en pacientes en diálisis en la regulación del apetito y en otras complicaciones como las cardiovasculares implicadas en la patogenia del síndrome MIA (malnutrición, inflamación y ateroesclerosis).

4.- Estudiar las interacciones entre trastornos del metabolismo hidrocarbonado, medido a través de marcadores en suero y expresión genética en tejido adiposo abdominal, con los trastornos en la regulación del apetito en pacientes en diálisis y con el síndrome MIA.

5.- Investigar el efecto del acetato de megestrol y del infliximab (anti-TNF-α) sobre el apetito y el estado nutricional de pacientes en diálisis.

6.- Todo ello, con el objetivo final de proporcionar argumentos que puedan sustentar como confluente la hipótesis de que la via del triptofano-serotonina en

hipotálamo unifica y es la que mejor explica el conjunto del entramado de factores reguladores del apetito conocidos hasta la actualidad.

4.- Pacientes y Métodos

Los pacientes renales incluidos en las diferentes partes del estudio son detallados en cada uno de los capitulos de resultados. En nuestra relación profesional con ellos siempre ha primado la preocupación por su estado en gereral y por la regulación de su apetito en particular. Para ello, hemos explicado en cada ocasión y a cada paciente el propósito de la investigación realizada, contando siempre con su complicidad. El objetivo constante ha sido contar con ellos para profundizar en conceptos tratados hasta ahora un tanto superficial y simplísticamente. Y el último objetivo fue modificar con conocimiento el abordaje del problema nutricional que tan negativamente afecta a los pacientes renales en diálisis.

Todos los materiales y métodos utilizados en esta tesis se describen en los apartados correspondientes de cada uno de los capítulos de resultados.

5.- Resultados

Capitulo 5.1. Estimación del impulso para comer. La inflamación y su influencia.

"La infección por *helicobacter pylori*: una nueva causa de anorexia en pacientes en diálisis peritoneal".

Aguilera A, Codoceo R, Bajo MA, Díez JJ, Del Peso G, Pavone M, Ortíz J, Váldez J, Cirugeda A, Fernandez-Perpen A, Sánchez-Tomero JA, Selgas R

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** Este trabajo responde a los objetivos 1 y 2.*

* Trabajo ganador de los premios:

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2.- Ganador del **Premio a la investigación en Nefrología clínica,** (Fundación Iñigo Alvarez de Toledo. Premio Reina Sofía de la investigación). Septiembre 2002.

La infección por *helicobacter pylori*: una nueva causa de anorexia en pacientes en diálisis peritoneal.

La infección por *helicobacter pylori (HP)* ha sido frecuentemente encontrada en pacientes en diálisis. Las infecciones crónicas inducen sobreproducción de sustancias pro-inflamatorias. La inflamación ha sido asociada con caquexia y anorexia. En este estudio exploramos la relación entre la infección por *HP* anorexia y malnutrición en pacientes en diálisis peritoneal (DP).

Pacientes y métodos. El estudio incluyo 48 pacientes clínicamente estables en DP que fueron divididos en 4 grupos: *HP* (+) con anorexia (grupo I, n=12); *HP* (+) sin anorexia (grupo II, n=4), *HP* (-) con anorexia (grupo III, n=5), y *HP* (-) sin anorexia (grupo IV, n=27). La infección por *HP* fue diagnosticada por el test del aliento. Anorexia fue evaluada usando una entrevista personal y una escala de motivación a comer o escala análoga visual (EAV). La EAV incluyo 5 preguntas que deben responderse antes y después de comer. Las preguntas son: deseo de comer, hambre, sensación de vacío y plenitud, cantidad de comida que comería y placer de comer. También determinamos marcadores bioquímicos de nutrición e inflamación.

Resultados. El grupo I mostró basalmente un bajo escore para el deseo de comer, hambre, sensación de vacío y plenitud futura consumición y el placer de comer. Ellos también mostraron bajo nº de linfocitos, prealbumina, transferrina, albúmina sérica, nPCR y función renal residual. Además, el mismo grupo mostró niveles elevados de proteína-C- reactiva (PCR) y más sensación de plenitud que el resto de los pacientes. En la serie total, encontramos una correlación linear estadísticamente significativa entre marcadores nutricionales

y EAV: albúmina con el deseo de comer antes de tomar la comida (r=0.38, p<0.05), y prelabumina con la misma variable (r=0.41, p<0.05) y negativa con la sensación de hambre después de comer (r=-0.35, p<0.05). Correlaciones negativas fueron encontradas entre la albúmina y la sensación de plenitud antes de comer (r=-0.45, p<0.01), y la misma variable con la prealbumina (r=-0.39, p<0.05). Una correlación negativa también observada entre PCR y albúmina (r=-0.35, p<0.05), y la misma variable con la prealbumina (r=-0.36, p<0.05). De manera similar, la PCR muestra una correlación negativa con el deseo de comer antes de comer (r=-0.38, p<0.05) y el deseo de comer después de comer (r=-00.45, p<0.01). Después de la erradicación del *HP*, el grupo I mostró una mejoría muy importante del estado nutricional y de la EAV en casi todas las preguntas. Simultáneamente, la PCR disminuyo. Muy importantes diferencias fueron encontradas antes y después de la erradicación del *HP*, nº de linfocitos (1105 ± 259.4 vs. 1330.8 ± 316 células/mm3, p<0.05), nPCR (0.9 ± 0.16, vs. 1.07 ± 0.3 g/kg/día, p<0.05), prealbúmina (26.7 ± 6.5 vs. 33.9 ± 5.6 mg/dL, p<0.01), albúmina (3.48 ± 0.3 vs. 3.67 ± 0.35 g/dL, p<0.05), PCR (1.16 ± 1.14 vs. 0.88 ± 1.2 mg/dL, p=0.054), deseo de comer antes de comer (56.6 ± 6.8 vs. 72.2 ± 4, p<0.001), deseo de comer después de comer (5.4 ± 2.6 vs. 12.3 ± 2, p<0.01), hambre antes de comer (55.4 ± 5.4 vs. 73.1 ± 4.6, p<0.001), hambre después de comer (5.8 ± 2.9 vs. 11 ± 4, p<0.01), sensación de plenitud antes de comer (36.6 ± 10.3 vs. 18.7 ± 8.8, p<0.001), deseo de consumición futura (5 ± 4.7 vs. 17.5 ± 18, p<0.05), y en el placer de comer (61 ± 5.3 vs. 74.1 ± 4.1, p<0.001).

Conclusión. La infección por *HP* se asocia con anorexia, inflamación y malnutrición en pacientes en DP. La erradicación del *HP* mejoro

significativamente este síndrome. La función renal residual tiene un efecto protector sobre la preservación de apetito mas que la dosis de diálisis. El presente estudio soporta la hipótesis de la participación de la inflamación en la patogénesis de la malnutrición en pacientes en DP.

Capitulo 5.2. Péptidos reguladores del apetito y el impulso a comer

"Niveles plasmáticos de ghrelina y apetito en pacientes en diálisis peritoneal".

Aguilera A, Cirugeda A, Amair R, Sansone G, Codoceo R, Bajo MA, del Peso G, Díez JJ, Sánchez-Tomero JA, Selgas R.

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** Este trabajo responde a los objetivos 1 y 2.*

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Niveles plasmáticos de ghrelina y apetito en pacientes en diálisis peritoneal.

Anorexia asociada a malnutrición es una complicación severa que aumenta la mortalidad en pacientes en diálisis peritoneal (DP). La ghrelina es una hormona orexigena recientemente descubierta con acción sobre el cerebro y estómago. Analizamos la posible relación entre ghrelina y la regulación del apetito en 42 pacientes en DP, además de otros orexigenos como el neuropeptido Y (NPY) y el óxido nítrico en forma de nitrato (NO3), y anorexigenos como la CCK, leptina, péptido insulina trópico dependiente de la glucosa (GIP) y el factor de necrosis tumoral alfa (TNF-α). Todos fueron determinados en plasma. La motivación a comer fue evaluada usado la escala análoga visual (EAV). Los pacientes fueron divididos en 3 grupos, pacientes con anorexia (n=12), obesidad asociada a alta ingesta (n=12) y los que no presentaban desordenes en la conducta alimenticia (n=18). Incluimos también 10 sujetos voluntarios sanos. Le media de niveles de ghrelina fue alta (3618.6 ± 1533 mg/mL), con 36 pacientes mostrando valores arriba del rango normal (<2600 mg/mL). Los pacientes con anorexia presentaron mas baja ghrelina, y NPY acompañado de niveles mas altos de péptido-C, CCK, interleuquina-1 (IL-1), TNF-α y GIP, que el resto de los pacientes. Estos mismos pacientes también presentaron saciedad temprana, bajo deseo y placer al comer medida por EAV y una baja ingesta medida por una encuesta dietética.

Encontramos una correlación positiva entre ghrelina y albúmina ($r=0.43$, $p<0.05$), prealbúmina ($r=0.51$, $p<0.05$), transferrina ($r=0.4$, $p<0.05$), hormona del crecimiento (GH) ($r=0.66$, $p<0.01$), NO3 ($r=0.36$, $p<0.05$) y motivación a comer (VAS). Al mismo tiempo, encontramos una relación negativa entre

ghrelina y GIP (r=-0.42, p<0.05), insulina (r=-0.42, p<0.05), leptina (r=-0.45, p<0.05) y CCr: (r=-0.33, p=0.08, NS). La ghrelina no presento relación con el Kt/V o con CCK y citoquinas.

Conclusión: La ghrelina esta elevada en pacientes en DP. Pacientes uremicos con anorexia mostraron niveles relativamente bajos de ghrelina que los obesos o los que no tenía trastornos del apetito. El papel de la ghrelina en la modulación del apetito esta alterada en pacientes uremicos en DP, este desorden es posiblemente asociado a desordenes en el metabolismo de insulina y GH.

Capitulo 5.3. Selectividad del apetito y peptidos regualdores

“Trastorno en la conducta alimenticia en la uremia: una cuestión de balance en la regulación del apetito”.

Aguilera A, Codoceo R, Bajo MA, Iglesias P, Díez JJ, Barril G, Sigarran C, Alvarez V, Celdilla O, Fernández-Perpen A, Montero A, Selgas R.

Semin Dial 2004; 17: 44-52.

** Este trabajo responde a los objetivos 1, 2, 3 y 6. Aunque es básicamente una revisión con formulación de hipótesis también contiene resultados originales.*

* Trabajo presentado como comunicación oral en varios congresos internacionales: USA y UK. Conferencista invitado al 9th world conference of clinical nutrition (9º congreso mundial de nutrición). Londres, Junio 24-26, 2002. Conferencia: “uremic anorexia, pathophysiology and management”

Trastorno en la conducta alimenticia en la uremia: una cuestión de balance en la regulación del apetito.

Los trastornos en el apetito son una complicación frecuente del síndrome urémico que puede contribuir a malnutrición en pacientes en diálisis. Los datos que sugieren que la anorexia urémica puede ocurrir con y sin acumulo de grasa abdominal y visceral a pesar de una baja ingesta. Esta forma de obesidad (con baja ingesta y malnutrición) es más común en pacientes diálisis que obesidades con elevada ingesta. Este trabajo revisa el conocimiento actual sobre los mecanismos responsables de regular el apetito en condiciones normal es y en el estado urémico. La anorexia en pacientes en diálisis ha sido históricamente considerada como un signo de toxicidad de la uremia debido a una diálisis inadecuada, tal como lo interpretan las hipótesis de las medianas moléculas, KT/V o la de los picos y otras. Nosotros proponemos la hipótesis del triptofano serotonina, basado en que la uremia induce un desorden en el perfil de amino ácidos, con baja concentración de amino ácidos largos neutros y principalmente ramificados de cadena corta con un nivel elevado de triptofano. Esto condiciona una elevada tasa de triptofano capaz de cruzar la barrera hemato-encefálica y sintetizar serotonina, el mayor inhibidor del apetito. La inflamación puede también juega un rol en la génesis de la anorexia y malnutrición. Por ejemplo, infecciones silentes por helicobacter pylori pueden ser la fuente de citoquinas con acción caquetizante. Su erradicación mejora el apetito y la malnutrición.

La evaluación del apetito debe considerar aspectos sociales y culturales. Los pacientes ureicos muestran una tendencia universal a carbohidratos y rechazo a carne roja comparado con la población normal medida a través de la encuesta de preferencias alimenticias utilizada por Dobell E, et al (SD53). En

contraposición la ingesta de carnes blancas fue menos problemática. Los pacientes urémicos también tuvieron una marcada atracción por los cítricos y sabores fuertes en general. Las preferencias y rechazos alimenticios han sido relacionados a la predominancia de algunos peptidos moduladores del apetito. Niveles elevados de CCK (anorexigeno) son asociados con saciedad temprana a carbohidratos, y neuropeptido Y (orexigeno) con repetidas ingesta alimenticia. En pacientes urémicos pueden contribuir al acumulo de grasa abdominal sin aumento del apetito, el estado hiperinsulinico, la leptina, IGF-I, ácidos grasos, desordenes en los receptores a de la insulina, lipoprotein-lipasa, la proteina-2 mitocondrial desacoplada y receptores β-adrenergicos. Esta obesidad con elevado indice de masa corporal (BMI) es frecuente y falsamente asociado a un adecuado estado nutricional.

Para evaluar el impulso a comer, nosotros utilizamos la escala análoga visual (EAV) (Hill J, et al), en 52 pacientes en diálisis en comparación con 104 voluntarios sanos de de los cuales 10 fueron incluidos en este estudio. Estos fueron contrastados con la población estudiada. Fuimos capaces de establecer los criterios diagnósticos de la anorexia uremica:

1.- EAV antes de comer < 60 mm, con al menos 4 horas de ayuno.

2.- El otro criterio fue baja ingesta alimenticia medida por una encuesta dietética < 30 kcal/kg/día.

3.- criterios de malnutrición definidos por los DOQI (SD64)

Por el contrario, obesidad con elevada ingesta fue definida de acuerdo con los siguientes criterios:

1.- EAV > 60 mm

2.- alto BMI definido de acuerdo con la OMS.

3.- ingesta alimenticia elevada medida por una encuesta dietética >35 kcal/kg/día.

Finalmente, la regulación del apetito en la uremia es altamente compleja. Los desordenes en el tejido adiposo, en el tracto gastrointestinal y en los neuropeptidos, las sustancias retenidas o hiperproducidas como las moléculas pro inflamatorias y sus productos finales, así como cambios en el sistema nervioso juegan un rol en el control inadecuado del apetito en el estado uremico. La anorexia urémica puede ser explicada por un estado hiperserotoninergico derivado de una alta concentración de triptofano y bajos niveles de amino ácidos ramificados de cadena corta.

Capitulo 5.4. Inflamación, peptidos, apetito y estado nutricional.

"Niveles plasmáticos de anorexigenos (TNF-α, colecistoquinina) y orexigeno (neuropeptido Y) en pacientes en diálisis: su relación con parámetros nutricionales".

Aguilera A, Selgas R, Codoceo R, Picorline M, García P, Picornell M, Diaz C, Sanchez C, Bajo MA.

Nephrol Dial Transp 13: 1476-1483, 1998.

***** *Este trabajo responde a los objetivos 1 y 4*

* Es el primer estudio en poner de manifiesto la asociación entre factores inflamatorios y trastornos del apetito en pacientes en diálisis. El Síndrome MIA fue publicado 2 año mas tarde.

Niveles plasmáticos de anorexigenos (TNF-α, colecistoquinina) y orexigeno (neuropeptido Y) en pacientes en diálisis: su relación con parámetros nutricionales.

La malnutrición en pacientes en diálisis ha sido definitivamente relacionada a una elevada mortalidad. Pérdida persistente del apetito es uno de los síntomas mas frecuentes encontrados en estos pacientes. Varias sustancias producen anorexia y desordenes en el ciclo hambre-saciedad en varias enfermedades.

El objetivo de este estudio fue identificar el papel que juegan sustancias anorexigenas (TNF-α y CCK) y orexigenas (NPY) en la génesis de la anorexia y malnutrición en 55 pacientes clínicamente estables en diálisis peritoneal.

Resultados. Niveles plasmáticos elevados de TNF-α fueron encontrados en 41/42 pacientes (97.6%), media (70.5 ± 32.3 pg/ml). Pacientes con anorexia (n=11) o con anorexia acompañada de nauseas y vómitos (n=5) tenían los niveles más altos de TNF-α que aquellos que no tenían síntomas (75.9 ± 34 vs. 52.1 ± 24.5 pg/ml, $p<0.05$). Ocho pacientes diagnosticados de enfermedad ácido péptica mostraron los niveles más altos de TNF-α (87.2 ± 24.3 pg/ml) que 30 pacientes sin enfermedad ácido péptica (63.6 ± 30.5 pg/ml, $p<0.05$). TNF-α mostró una correlación negativa, estadísticamente significativa con la proteína transportadora del retinol (RBP) ($r=-0.37$, $p<0.05$), y el pH venoso ($r=-0.4$, $n=42$, $p<0.01$); así mismo, niveles de TNF-α > 65 pg/ml estaban inversamente relacionados con transferían, colesterol, urea, y CCK. Pacientes con prealbumina < 30 mg/dL, BMI < 30 kg/m2, nPCR <1.1 g/kg/día y KT/V de urea < 2.2 mostraron niveles elevados de TNF-α. Aquellos pacientes quienes han

estado en esta modalidad de tratamiento por largo tiempo también mostraron los niveles más elevados de TNF-α.

En relación a la CCK, fueron encontrados los niveles elevados en 38 de 45 pacientes (84%), media 45.9 ± 32.3 pg/ml. No encontramos diferencia en los niveles de CCK entre pacientes que sufren anorexia vs. los que presentan apetito normal. En relación a la asociación entre CCK y el estado nutricional, encontramos una asociación directa entre CCK y albúmina, fibronectina, triglicéridos, ácido fólico, y nPCR en no diabéticos). Aunque CCK muestra en efecto anorexigeno, esta asociación directa con marcadores nutricionales puede deberse a una estimulación anormal del feed-back de la CCK-glucosa vía tripsina debido a la absorción peritoneal constante de glucosa. Esto podría sugerir que la CCK puede ser un marcador nutricional temprano (ingesta).

Los niveles plasmáticos de NPY fueron normales en 33 pacientes, altos en 6 y bajos en 11. Los pacientes con anorexia presentaron niveles más bajos de NPY que el resto. NPY < 50 pg/ml fue positivamente asociado con transferrina, prealbúmina, RBP, nPCR y KT/V de urea.

Encontramos una correlación negativa entre NPY y TNF-α ($r=-0.42$, $n=41$, $p<0.01$). No hubo relación significativa entre la función renal residual y los niveles en suero de estos 3 peptidos.

Conclusión: nuestros datos sugieren que los niveles elevados de TNF-α y bajos de NPY están asociados a anorexia. Niveles plasmáticos elevado de TNF-α, bajos de CCK y NPY están relacionados con pobre estado nutricional. Estudios futuros sobre estas sustancias circulantes son requeridos.

Capitulo 5.5. Inflamación, nutrición y riesgo cardiovascular.

“El factor de necrosis tumoral alpha como una toxina urémica: su correlación con neuropatía, hipertrofia ventricular izquierda, anemia, hipertrigliceridemia en pacientes en diálisis peritoneal”.

Espinoza M, **Aguilera A**, Auxiliadora Bajo M, Codoceo R, Caravaca E, Cirugeda A, del Peso G, Hevia C, Selgas R.

Adv perit Dial 1999; 15: 82-86.

***** *Este trabajo responde al objetivos 3, 4*

* Plantea la posibilidad de utilizar medicamentos anti-TNFα como tratamiento de la malnutrición y enfermedad vascular en pacientes en diálisis. Este trabajo ha sido presentado en varios congresos nacionales e internacionales

El factor de necrosis tumoral alpha como una toxina urémica: su correlación con neuropatía, hipertrofia ventricular izquierda, anemia, hipertrigliceridemia en pacientes en diálisis peritoneal.

El factor de necrosis tumoral alpha (TNF-α) es habitualmente excretado por los riñones. En pacientes en diálisis, este se acumula. TNF-α ha sido implicado en la patogénesis de la malnutrición, neuropatía diabética y resistencia a la eritropoyetina.

Estudiamos los niveles plasmáticos de TNF-α en 49 pacientes clínicamente estables en diálisis peritoneal (DP), con el objetivo de correlacionar estos niveles con la presencia y severidad de la neuropatía periférica, miocardiopatía hipertrófica, y anemia. Medimos el KTV de urea la función renal residual (FRR), marcadores nutricionales y realizamos una bioquímica general. La media de TNF-α fue (67 ± 32 pg/mL, rango 18.1-156.3 pg/mL, normal: 3-20). No encontramos correlación entre TNF-α y KTV, pero si encontramos correlación entre TNF-α y FRR medida por CCr (1.5 ± 1.7 vs. 3.9 ± 2.6 mL/min, $p<0.01$). Los niveles de TNF-α fueron mas altos en los pacientes con HVI (70.4 ± 32 vs. 38.5 ± 20.8 pg/mL, $p<0.05$). Los pacientes con HVI severa presentarón la mas baja FRR. Encontramos una relación estadísticamente significativa entre los niveles de TNF-α y la dosis de EPO ($r=0.41$, $p<0.05$). Los pacientes con hipertrigliceridemia tomando hipolipemiantes orales mostraron una correlación positiva con el nivel de triglicéridos ($r=0.7$, $n=14$, $p<0.05$).estos datos sugieren que el acumulo de TNF-α pueden contribuir al desarrollo y mantenimiento de complicaciones neurologicas, hematologicas y cardícas del síndrome uremico. La perdida de la función renal residual condiciona un aumento de los niveles de

TNF-α. Estos datos dan y añaden soporte a la idea de que el TNF-α puede ser considerado como una toxina urémica.

Capitulo 5.6. Inflamación, nutrición y disfunción endotelial.

5.6.1.- "El síndrome malnutrición-inflamación se asocia con disfunción endotelial in pacientes en diálisis peritoneal".

Aguilera A, Sanchez-Tomero JA, Bajo MA, Ruiz-Caravaca ML, Alvarez V, del Peso G, Herranz A, Cuesta MV, Castro MJ, Selgas R.

Adv Perit Dial. 2003;19:240-5

***** *Este trabajo responde a los objetivos 3, 4*

* Plantea una asociación definitiva entre inflamación sistemica, disfunción endotelial y malnutrición. En definitiva entre los diferentes componentes del síndrome MIA. Refuerza además la posibilidad de utilizar medicamentos anti-TNFα como tratamiento de la enfermedad vascular en pacientes en diálisis.

* Este trabajo ha sido presentado en varios congresos nacionales e internacionales

El síndrome malnutrición-inflamación se asocia con disfunción endotelial in pacientes en diálisis peritoneal.

La disfunción endotelial con ateroesclerosis es una complicación reconocida de los pacientes uremicos. La hipoalbuminemia de los pacientes en diálisis peritoneal (DP) puede inducir estados de hipercoagulabilidad y un estado pro-aterogenico. En el presente estudio investigamos el papel jugado por el síndrome malnutrición-inflamación sobre marcadores de función endotelial en pacientes en DP. Medimos, marcadores nutricionales la tasa [normalizada de metabolismo proteico (nPCR), albumina, prealbumina, factor de crecimiento de insulina tipo I (IGF-I), transferrina y colesterol], marcadores de daño y función endotelial [activador del plasminogeno tisular (tPA), trombomodulina (TM), von Willebrand factor (vWF) y NO3 como representante del oxido nitrico (NO)], marcadores del estado de coagulabilidad [fibrinogeno y activador del inhibidor del plasminogeno 1 (PAI-1)], marcadores de inflamación [factor de necrosis alfa (TNF-α) y proteina-C-reactiva (PCR)], y otros factores asociados a daño endotelial [lipoproteina(a) [Lp(a)] y homocisteina (Hcy)]. Realizamos una prueba de función endotelial consistente en una prueba de oclusión venosa (OV) por 10 minutos. Los pacientes fueron divididos en 4 grupos de acuerdo con un score ateroesclerotico clínico (SAC).

Estudiamos 45 pacientes estables en DP. Basalmente, encontramos una correlación negativa entre albúmina y edad ($r= -0.54$, $p<0.05$), albúmina y vwF post-OV ($r=-0.54$, $p<0.05$), y albúmina con TM ($r=-0.36$, $p<0.05$), los cuales son marcadores de daño endotelial y factores pro-trombóticos. Una correlación positiva fue encontrada entre albúmina y NO3 post-VO ($r= 0.48$, $p<0.05$), indicando una alta capacidad vasodilatadora. PCR y TNF-α mostraron una

correlación linear positiva (r=0.5, p<0.01). Similarmente, TNF-α mostró una correlación linear positiva con marcadores de riego CV como el fibrinógeno (r=0.79, p<0.01), PAI (r=0.44, p<0.05), y Hcy (r=0.37, p<0.05). CCr mostró una correlación negativa con TM (r=-0.36, p<0.05). Los pacientes con albúmina < 4 g/dL mostraron una bajo tPA-ratio, bajo NO3, y alta PCR, TNF-α y Lp(a) que aquellos con albúmina > 4 g/dL [tPA ratio: 2.1 ± 1.56 (n = 29) vs. 2.6 ± 2.3 (n = 16), p < 0.05; NO3: 47 ± 27 μg/mL vs. 69 ± 33 micrograms/mL, p < 0.05; CRP: 1.8 ± 3 mg/dL vs. 1.1 ± 1.6 mg/dL, p < 0.05; TNF alpha: 44.4 ± 16 pg/mL vs. 36.6 ± 21.4 pg/mL, p < 0.05; Lp(a): 55 ± 39 mg/dL vs. 33 ± 21 mg/dL, p < 0.05]. Los pacientes con peor SAC mostraron niveles elevados de Hcy y baja albúmina. No encontramos relación entre la dosis de diálisis y la función endotelial.

En conclusión, el síndrome malnutrición-inflamación puede contribuir al síndrome de disfunción endotelial y, consecuentemente a procesos pro-trombóticos y pro-aterogénicos en pacientes en DP.

5.6.2.- “La inflamación sistémica induce disfunción endotelial en pacientes tratados con diálisis peritoneal”.

Abelardo Aguilera, Eddy Velásquez, María A Bajo, Maria L Ruiz-Caravaca, Mario Pavone, Victoria Martínez, Gloria del Peso, Angel Herranz, Maria V. Cuesta, Manuel López-Cabrera, Rafael Selgas.

** Este trabajo responde al objetivos 3, 4*

La inflamación sistémica induce disfunción endotelial en pacientes tratados con diálisis peritoneal.

La disfunción endotelial (DE) asociada a ateroesclerosis es una complicación frecuente de los pacientes uremicos. Recientemente se ha sugerido la importancia de la inflamación en la fisiopatología de la DE. El objetivo de este estudio es analizar el papel jugado por la inflamación sistemica como iniciador de la DE en pacientes en diálisis peritoneal (DP).

Métodos. Todos los pacientes de nuestra unidad de DP fueron seguidos durante 15 meses. Medimos, marcadores nutricionales, inflamatorios (proteína-C-reactiva (PCR), TNF-α y la molécula de adhesión vascular tipo-I (VCAM) y de función endotelial, de forma basal y al aparecer un proceso inflamatorio sistémico (IS). 17 pacientes fueron finalmente incluidos debido a que presentaron elevación de la PCR asociado a varias etiologías (4 sufrieron infección silente por *HP*, 4 infecciones respiratorias superiores, 2 sobre crecimiento bacteriano intestinal y causas desconocidas en 7). Estos fueron comparados con un grupo control (GC) con 12 pacientes que no sufrieron IS.

Realizamos un test de oclusión venosa (VOT) para estimular el endotelio induciendo isquemia y ectasis durante 10 minutos con el esfigmomanómetro insuflado (brazo derecho). Las muestras de sangre fueron tomadas antes y después del VOT. La capacidad fibrinolitica endotelial fue medida a través del activador del plasminogeno tisular (tPA) y el inhibidor del activador (PAI). Los marcadores de daño endotelial medidos fueron el von Willebrand factor (vWF), la trombomodulina (TM), y el oxido nítrico (NO). Los marcadores de riego cardiovascular (CV), fibrinogeno, lipoproteína(a) y homocisteina (Hcy). Los marcadores VCAM-1, factor de crecimiento vascular (VEGF), factor de

transformación tipo β (TGF-β) y factor de crecimiento derivado de las plaquetas (PDGF). Después de un tiempo variable de seguimiento en los pacientes estudiados la PCR y el TNF-α aumento.

Resultados. Después de sufrir IS encontramos una disminución en la albúmina, tPA-ratio (post VOT/pre-VOT) y NO_3-ratio. PAI, TM, Lp(a) y TGF-β aumento. Encontramos una correlación positive entre Hcy y PDGF, sugiriendo a pro-aterogenico. GC no sufrió ninguna modificación.

Conclusión: En pacientes en DP la IS induce DE medida a través marcadores de daño endotelial. La elevación de citoquinas proinflamatorias esta estrechamente relacionada con elevación de mediadores pro-cogulantes y pro-ateroescleroticos en plasma.

5.6.3.- "La erradicación de *Helicobacter pylori* mejora la malnutrición, la inflamación y la arterisoclerosis en pacientes en diálsis peritoneal".

Abelardo Aguilera, Rafael Selgas, Rosa Codoceo, M. Auxiliadora Bajo, Juan J. Diéz, Pedro Iglesias, Rosa Martin, Olga Celadilla, Maria J Castro, Camen Mansilla, Agustin Montero.

***** *Este trabajo responde al objetivos 3, 4 y fue ganador de los premios:*

1.- Ganador al **mejor trabajo de investigación** (best abstract-research). International peritoneal dialysis congress. Montreal. Canada. Junio 2001

2.- Ganador del **Premio a la investigación en Nefrología clínica,** (Fundación Iñigo Álvarez de Toledo. Premio Reina Sofía de la investigación). Septiembre 2002.

* Este estudio estable una relación causa-efecto entre la presencia de una infección crónica silente con gran producción de citoquinas y el síndrome MIA. La erradicación del *HP* resulta en una mejoría sustancial de los marcadores del MIA. Resalta además la importancia de la función renal residual como factor protector, mas que la dosis de diálisis.

La erradicación de *Helicobacter pylori* mejora la malnutrición, la inflamación y la arterisoclerosis en pacientes en diálsis peritoneal

La infección por *Helicobacter pylori (HP)* ha sido detectada frecuentemente en pacientes en diálisis. Las infecciones crónicas inducen producción de citoquinas las cuales son aclaradas pobremente por insuficiencia renal. Estas citoquinas tienen efectos sistémicos y catabólicos.

Estudiamos el efecto de la infección y erradicación del *HP* sobre los niveles séricos de citoquinas, estado nutricional y sobre marcadores de función endotelial (FE) en pacientes en diálisis peritoneal (DP).

Métodos. La infección por *HP* fue diagnosticad a través de test del aliento. Medimos, pre y post erradicación de *HP*, marcadores nutricionales, bioquímicos, ingesta alimenticia diaria y moduladores del apetito (orexigenos neuropeptido-Y, NPY, anorexigeno colecistiquinina, CCK). Secreción ácida en él estomago: pepsinogeno-I y II, y marcadores inflamatorios: proteina-C reactiva (CRP), niveles plasmáticos de TNF-α y IL-6. Y marcadores de función endotelial que fueron tomados pre- y post-oclusión venosa (VOT), se toma la tensión arterial (TA), se calculo la TA media (TAM), se insuflo el esfignomanametro a la TA en el brazo derecho durante 10 minutos y se tomaron muestras de sangre antes y después del VOT. El activador tisular del plasminogeno (tPA), NO3 (representando el oxido nítrico) y el activador del inhibidor del plasminogeno tipo-I (PAI).

Cuarenta y ocho pacientes en diálisis peritoneal clínicamente estables fueron divididos en 4 grupos de acuerdo a la presencia de infección por *HP* e ingesta alimenticia. Grupo I *HP* (+) y baja ingesta (<30 kcal/ kg/día, DOQI guías)

n=12. Grupo II, HP (+) e ingesta alimenticia normal, n=4. Grupo III, HP (-) y baja ingesta n=5. Grupo IV, HP (-) e ingesta normal n=27.

Valores de TNF-α, grupo I: 125 $\pm$ 85* pg/ml, grupo-II: 70.5 $\pm$ 25, grupo III: 60.5 $\pm$ 10*, y grupo IV 43.4 $\pm$ 5.4*, * <0.05. Con relación a la IL-6: grupo I: 34.2 $\pm$ 18* pg/ml. G 3.4 $\pm$ 7*, *<0.05. Grupo III: 11.1 $\pm$ 7.9*, *<0.05 y grupo IV 1.02 $\pm$ 0.65 *, * <0.05. La función renal residual fue significativamente mas alta en el grupo IV (4.8 $\pm$ 1.6* ml/min) que el grupo I (2.7 $\pm$ 2.3*, p<0.05). Después de la erradicación del HP en el grupo-I mejoro nutricional y bioquímicamente con aumento del NPY. Los marcadores inflamatorios y de secreción ácida disminuyeron. La función endotelial también mejoro. El grupo control con 10 voluntarios no renales infectados por *HP*, experimentaron una leve mejoría en marcadores nutricionales, después de la erradicación del *HP* que se asocio además con mejoría en parámetros inflamatorios y el los marcadores de secreción ácida del estomago.

Conclusión: La infección por *HP* induce sobre producción de citoquinas, que se asocia a malnutricición, inflamación sistémica y ateroesclerosis en pacientes en DP. La erradicación del *HP* normalizo la secreción ácida del estomago, disminuyo la inflamación y mejoro el estado nutricional y la disfunción endotelial.

Capitulo 5.7.- Tejido adiposo, estado nutricional y riego cardiovascular.

"La leptina como marcador nutricional y de riesgo cardiovascular en pacientes en diálisis peritoneal".

Aguilera A, Bajo MA, Rebollo F, Díez JJ, Roca A, Díaz C, Paiva A, Codoceo R, Selgas R.

Adv Perit Dial 2002; 18: 212-217.

***** *Este trabajo responde a los objetivos 3, 4*

* Este trabajo ha sido presentado en varios congresos nacionales e internacionales

La leptina como marcador nutricional y de riesgo cardiovascular en pacientes en diálisis peritoneal.

Anorexia y malnutrición proteica, ocasionalmente asociado con obesidad son frecuentemente encontrados en pacientes en diálisis peritoneal (DP). Ambos son reconocidos factores de riesgo cardiovascular (CV), de morbilidad y mortalidad. La leptina es producida por lo adipositos y regula la masa grasa induciendo saciedad central. La leptina se acumula en ele estado urémico.

Nosotros analizamos la relación entre los niveles plasmáticos de leptina, estado nutricional, obesidad, factores de riesgo CV y aterosclerosis en pacientes en DP. La leptina fue determinada usando un anticuerpo policlonal (RIA. Linco Research, St. Louis, MO, USA). El rango normal fue de 1-7.8 ng/mL.

Estudiamos 38 pacientes en DP. La media de leptina plasmática fue: 59.1 ± 57.5 ng/mL (elevado en 32 pacientes). Las mujeres (n=21) presentaron niveles más elevados de leptina que los hombres (80.4 ± 60 vs. 32.3 ± 43.3 ng/mL, $p<0.01$), a pesar que ambos grupos tenían igual BMI. Encontramos una correlación directa estadísticamente significativa entre leptina y BMI ($r=0.7$, $p<0.01$) y el pliegue tricipital ($r=0.77$, $p<0.01$). Los niveles de leptina y él aclaración de Cr no mostraron correlación. Independientemente del BMI, niveles elevados de leptina fueron asociados con parámetros de riesgo CV (estudios Framingham) incluyendo, triglicéridos < 150 (n=29) vs. >150 mg/dL (44.2 ± 53.2 vs. 80 ± 58.4 ng/mL, $p<0.05$), colesterol <250 (n=28) vs. >250 mg/dL (50 ± 55.6 vs. 84.7 ± 57.7 mg/dL, $p<0.05$), y con la presencia o ausencia de hipertrofia ventricular izquierda (HVI) (68.8 ± 60, n=30, vs. 29.5 ± 23.7, n=5, $p<0.05$). Los pacientes fueron clasificados en 2 grupos de acuerdo al escore ateroesclerotico clínico (EAC). Diecinueve pacientes mostraron alto EAC y altos

niveles de leptina que el resto (82.4 ± 65.7 vs. 35.8 ± 36.6 ng/mL, $p<0.05$). Doce pacientes con anorexia presentaron bajos noveles de leptina que aquellos con apetito normal (19.2 ± 15.8 vs. 91.3 ± 58.8 ng/mL, $p<0.001$). en no-obesos (BMI < 25 y CCr < 3 mL/min, n=14), la leptina presento una correlación estadística, directa significativa con marcadores nutricionales incluyendo la albúmina ($r=0.63$, $p<0.05$), transferrina ($r=0.4$, $p<0.05$), colesterol ($r=0.65$, $p<0.05$) y triglicéridos ($r=0.6$, $p<0.05$). Finalmente, los niveles plasmáticos de leptina estaban notablemente elevados en la población en DP, indicando un aumento en la producción posiblemente por hiperinsulinismo crónico, retención urémica o ambas. A través de un análisis multivariante confirmamos la asociación entre leptina y sexo, leptina y BMI, y leptina >40 ng/mL y sexo e HVI. Todos estas características sugieren que los niveles plasmáticos de leptina pueden ser considerados como marcador de riego CV y de ingesta de alimentos en pacientes no obesos sin inflamación.

Capitulo 5.8.- Eje anabolizante y uremia

“Modulación colinergica de la respuesta de la hormona del crecimiento a la hormona liberadora de la hormona del crecimiento en pacientes uremicos en diálisis peritoneal”.

Diez JJ, Iglesias P, Selgas R, Bajo MA, **Aguilera A**.

Clin Endocrinol 2000; 53(5):587-93

** Este trabajo responde a los objetivos 3 y 4*

* Este trabajo ha sido presentado en varios congresos nacionales e internacionales

Modulación colinergica de la respuesta de la hormona del crecimiento a la hormona liberadora de la hormona del crecimiento en pacientes uremicos en diálisis peritoneal.

Los neurotransmisores colinergicos hipotalamicos juegan el mayor papel en la regulación de la secreción de hormona del crecimiento (GH). Piridostignina, un inhibidor de la colinesterasa, es capaz de disminuir el tono samatostatinergico hipotalamico y liberar GH en sujetos normales. El bloqueo de los receptores muscarinicos con pirenzepina bloquea la liberación de GH en varias condiciones clínicas. Sin embargo, hay poca información disponible sobre el papel jugado por la vía colinergica central en la regulación de la GH en pacientes uremicos.

Objetivo: nuestro objetivo fue medir la respuesta de la GH a la hormona liberadora de GH después de pretratar con piridostigmina y pirenzepina en un grupo de pacientes uremicos en diálisis peritoneal (DP).

La respuesta de la GH de los pacientes tratados con eritropoyetina recombinante humana (EPO) se comparo con pacientes sin este tratamiento.

Diseño: investigamos 14 hombres en DP y 9 voluntarios sanos. Todos los sujetos fueron sometidos a 3 test endocrinologicos. Cada sugeto recibio hormona liberadora de GH (100 μg, IV en bolus al minuto “o”). 16 minutos después de la inyección de hormona liberadora de GH fue dado un placebo oral, pyridostigmine (120 mg), ó pirencepina (100 mg)

Determinaciones: las muestras de GH fueron colectadas a -60, 0, 15, 30, 45, 60 y 90 minutos. La respuesta hormonal secretoria fue estudiada en función al tiempo (área bajo la curva, ABC) y a tiempos independientes (valores pico).

Resultados: basalmente, la concentración de GH fue similar en los pacientes que en los controles. La respuesta de la GH a placebo mas hormona liberadora de GH fue también comparable (pico 26.6 ± 3.8 vs. 33.2 ± 4.4 mU/l, ABC 28.2 ± 3.4 vs. 27.8 ± 4.6 mU/h/l). La administración de piridostignina indujo una potenciación significativa de la respuesta de la GH a su hormana liberadora que también fue comparable en pacientes (pico 43.2 ± 5.2 mU/l, ABC 47.6 ± 6.0 mU/h/l; $P < 0.01$), que en controles (pico 79.2 ± 8.6 mU/l, ABC 78.0 ± 9.4 mU/h/l; $P < 0.01$). Sin embargo, el incremento en el pico de GH y ABC fue significativo, más alto en controles que en los pacientes.

La administración de pirenzepina indujo una abolición de la GH después de estimulación con su hormona liberadora, en pacientes (pico 5.4 ± 2.6 mU/l, ABC 6.0 ± 2.4 mU/h/l; $P < 0.01$) y en voluntarios sanos (pico 3.8 ± 0.6 mU/l, ABC 4.0 ± 0.4 mU/h/l; $P < 0.05$).

La respuesta a la piridostignina mas hormona liberadora de GH y pirenzepina mas la misma fue similar en pacientes con terapia crónica con EPO y sin ella.

Conclusion: nuestros resultados suguieren que la regulación colinergica de la liberación de GH esta preserva en la unremia y en pacientews en DP. El incremento significativamente bajo en la respuesta a la GH por su hormona liberadora (piridostignina), sugiere que la estimulación del tono colinergico es atenuado en pacientes en relación a los controles. La terapia a largo plazo con EPO no parece afectar la respuesta de la GH a su estimulación o bloqueo.

Capitulo 5.9.- Tejido adiposo y peptidos reguladores del apetito.

“Las alteraciones en la liberación de los péptidos moduladores del apetito explican los trastornos en la conducta alimenticia de los pacientes en diálisis peritoneal”.

Aguilera A, Codoceo R, Bajo MA, Caravaca E, Diez JJ, Jara MC, del Peso G, Herranz A, Grande C, Selgas R. *En vías de publicación*.

** Este trabajo responde al objetivo 4.*

* Plantea una interesante conección entre el tejido graso, la expresión genetica de adipoquinas, resistencia hidrocarbonada, factores inflamatorios y los trastornos en la liberación de peptidos moduladores del apetito.

* He sido presentado en varios congresos nacionales e internacionales como comunicación oral.

Las alteraciones en la liberación de los péptidos moduladores del apetito explican los trastornos en la conducta alimenticia de los pacientes en diálisis peritoneal

La malnutrición es una complicación frecuente en paciente en diálisis peritoneal (DP), los trastornos del apetito conducen a malnutrición especialmente la anorexia, y la obesidad a malnutrición de tipo protéico. Este estudio analiza los trastornos en la conducta alimenticia de 42 pacientes en DP, 12 anoréxicos, 12 obesos, 18 sin trastornos del apetito y 10 controles sanos. El impulso a comer se analizo con la escala análoga visual (EAV), los pacientes anoréxicos presentaron mayor valores en el score de saciedad antes y después de comer, menor deseo y placer al comer. El neuropéptido Y (NPY) el más poderoso orexígeno conocido y la colecistoquinina (CCK) (un potente anorexígeno), se correlaciono con este impulso. Los anoréxicos presentaron niveles mas altos de factores anorexígenos (insulina, glucosa, péptido C, CCK, IL-1, TNF-α y péptido inhibidor gástrico (GIP)), y niveles relativamente bajos de NPY. Por el contrario, los obesos presentaron niveles mas altos de NPY y bajos de anorexígenos. También encontramos desordenes en la liberación de péptidos tras estimulo alimenticio. Los anoréxicos presentaron un "pico" de CCK temprano y muy elevado, lo que explicaría la saciedad temprana que sufren estos pacientes en la EAV. Además la curva del NPY es los anoréxicos fue plana y descendió a niveles mas bajos de los basales 90 minutos después de comer, agravando la falta de apetito tardío. Los obesos presentaron dos "picos" de NPY, uno temprano (30 minutos) y otro tardío (90 minutos), que explica las ingestas abundantes y reiteradas. La curva de la insulina fue completamente paralela a la del NPY, mostrando el efecto de la insulina sobre

este péptido. La IL-1 se correlaciono con la insulina y el GIP, ambos son estimulantes de la insulina. La IL-1 ejerce su efecto anorexígeno vía CCK. Los uremicos sufren un sufren un síndrome de intolerancia hidrocarbonada con hiperinsulinemia. Este síndrome puede alterar el equilibrio normal de los pépticos moduladores del apetito, dando origen a trastornos en la conducta alimenticia. La leptina participo como anorexígeno solo de forma basal. El NO_3 como forma de determinar el oxido nítrico (NO) también fue determinado. Todos los pacientes en DP presentaron un descenso de los mismos 30 minutos después de comer. Todos los pacientes uremicos presentaron un rebote a los 90 minutos, elevando sus niveles, especialmente los obesos. Los controles disminuyeron efectivamente la curva de ghrelina después de comer.

Muestras de grasa abdominal fueron obtenidas durante la colocación de catéteres de DP o cirugías electivas. De tal manera que obtuvimos 5 muestras del grupo I, 6 del grupo II, 4 del grupo III y 4 del grupo control.

Determinamos la expresión génica de leptina, adiponectina y TNF-α por PCR cuantitativa. Todos los uremicos presentaron una sobre-expresión de TNF-α, la más elevada en los anoréxicos, seguido por los obesos y los uremicos asintomático. Un patrón inverso fue encontrado cuando se analizo la leptina y la adiponectina, donde los niveles más bajos fueron encontrados en los uremicos. En relación a la adiponectina los niveles mas bajos se encontraron en los obesos.

Finalmente: la conducta alimenticia en pacientes en DP es modulada al menos en parte, por pépticos con acción central y periférica que elevados de forma basal se liberan de anómalamente. Esto es debido a la retención renal que estos pépticos sufren, a la interacción de los mismos entre sí, a la

retención y sobre producción de sustancia que normalmente se producen en pequeñas cantidades (toxinas urémicas), y a la intolerancia hidrocarbonada del urémico que parece jugar un papel clave en la liberación de los péptidos.

Capitulo 5.10.- Intervenciones sobre el apetito y la inflamación

“Tratamiento de la anorexia y malnutrición con acetato de megestrol en pacientes en diálisis peritoneal”.

Costero O, Bajo MA, del Peso G, Gil F, **Aguilera A**, Ros S, Hevia C, Selgas R. *Adv Perit Dial*. *2004;20:209-12*

** Este trabajo responde al objetivo 5.*

Tratamiento de la anorexia y malnutrición con acetato de megestrol en pacientes en diálisis peritoneal.

La anorexia y malnutrición son complicaciones comunes y poderosos predoctores de mortalidad y morbilidad en pacientes en diálisis peritoneal (DP). El acetato de megestrol (AM) es un progestageno que ha demostrado que aumenta el apetito y el peso en pacientes con cáncer o síndrome de inmunodeficiencia adquirido. Con el objetivo de determinar si el AM puede beneficiar pacientes en DP, nosotros tratamos 32 pacientes con 160 mg diarios. El periodo de tratamiento fue 5.93 ± 5.12 meses (rango 1-23 meses). En el 68.8% de los pacientes, mejoró el apetito. La ganancia de peso fue estadísticamente al 3er mes (la media del peso inicial 66.5 ± 11.4 kg, a los 3 meses fue 68 ± 10.4 kg; $p<0.05$). Observamos un incremento no significativo de la albúmina sérica al 3er mes (inicial 3.44 ± 0.27 g/L, al tercer mes 3.54 ± 0.27, $p=0.45$). No observamos efectos secundarios. Nuestra experiencia sugiere que el tratamiento con 160 mg de AM diario en pacientes en DP indujo un aumento del apetito, albúmina sérica, y ganancia de peso en muchos pacientes, sin efectos secundarios.

6.- Discusión.

A lo largo de estos años pretendimos desarrollar una línea continua de investigación en los trastornos del apetito en los pacientes en diálisis, bajo la concienciación de que el primer paso para obtener un estado nutricional adecuado es tener una ingesta apropiada de alimentos. De tal manera, que nuestro primer objetivo fue establecer los factores de riesgo para presentar anorexia uremica y en el otro extremo, obesidad con bulimia. Fue así como en nuestros primeros trabajos determinamos que para sufrir anorexia uremica eran factores de riesgo, presentar anuria, largo tiempo en diálisis, enfermedades crónicas potencialmente caquetizantes como insuficiencia cardiaca o enfermedades hepáticas, infecciones de repetición y la cantidad de medicamentos ingeridos a lo largo del día. El sexo femenino y la dosis de diálisis se quedaron al borde de la significación estadística (resultados capitulo del I al IV). Con relación a la dosis de diálisis, a pesar que tradicionalmente la anorexia ha sido considerada como un signo de toxicidad de la uremia (CANUSA) (36), en el análisis estadístico pesó más la función renal residual. Resultados recientes de otros autores (37) refuerzan nuestras conclusiones.

Como factores de riesgo para sufrir obesidad/bulimia en pacientes en diálisis encontramos que la diabetes mellitus, el hiperinsulinismo basal, el peso elevado al entrar en diálisis, el sedentarismo y el tipo de diálisis (diálisis peritoneal) fueron determinantes para sufrir obesidad. De forma interesante, encontramos que el sufrir o haber sufrido hiperparatiroidismo secundario se asoció ligeramente a obesidad central. Este último hecho puede ser debido a una desregulación de los genes que regulan la distribución de grasa en el cuerpo (resultados capitulo 5.3).

De acuerdo con nuestra información esta es la primera vez que se establecen los factores de riesgo para sufrir trastornos de la conducta alimenticia en pacientes en diálisis. Además la importancia del apetito en esta población no ha sido reconocida hasta hace dos años (38).

Nuestro siguiente objetivo fue encontrar y validar una encuesta que nos sirviera para valorar y definir la intensidad del apetito en la población en diálisis. En este caso, utilizamos la escala análoga visual (EAV) publicada por Hill J, et al (39). Una de las razones por las que seleccionamos esta encuesta es porque existian validaciones previas en pacientes en diálisis, aunque no en nuestra población (16, 40, 41). Este instrumento fue de gran utilidad ya que resulta difícil evaluar un síntoma tan subjetivo como es el apetito, en especial si consideramos que involucra aspectos biológicos, sociales, culturales y religiosos. En el estado uremico la situación es aun más compleja ya que a los moduladores normales se añaden sustancias que se encuentran alteradas, bien por retención uremica o por trastornos en su liberación (33). A través de los diferentes estudios que realizamos, tanto transversales como seguimientos cortos, fuimos capaces de establecer los criterios para definir la anorexia uremica en nuestra población (resultados capitulo 5.3):

1.- EAV antes de comer < 60 mm, con al menos 4 horas de ayuno.

2.- Baja ingesta alimenticia medida por una encuesta dietética < 30 kcal/kg/día.

3.- Criterios de malnutrición definidos por los DOQI (42).

La obesidad con elevada ingesta fue definida de acuerdo con los siguientes criterios:

1.- EAV > 60 mm

2.- Alto BMI definido de acuerdo con la OMS.

3.- Ingesta alimenticia elevada medida por una encuesta dietética >35 kcal/kg/día.

En este estudio (Capitulo 5.3) evaluamos la conducta alimenticia de 52 pacientes en diálisis y la comparamos con 104 voluntarios sanos comparables con la población estudiada. En otros estudios que utilizamos la EAV incluimos 48 y 42 pacientes en diálisis respectivamente (resultados capitulo 5.1 y 5.2).

Sin embargo, existen otros instrumentos que se han utilizado con el mismo fin en pacientes en diálisis, como la *"visual analog ratings*", utilizada por Wright M et al. (17, 43).

Nuestro tercer objetivo fue indentificar el papel de algunas sustancias biológicamente activas que podrían retenerse o hiperproducirse en pacientes en diálisis, lo que podría deberse a ineficiencia de los métodos actuales de diálisis para eliminar moléculas con capacidad de dismunuir el apetito. Como ocurre con las citoquinas pro-inflamatorias que se liberan en grandes cantidades después que los polimorfonucleares entran en contacto con las membranas de diálisis.

La primera sustancia que investigamos fue el TNF-α por su reconocido efecto caquectizante y anorexigeno. Efectivamente, encontramos niveles elevados de esta citoquina en el 99% de los pacientes, sin asociación con la dosis de diálisis pero si con la función renal residual (resultados capitulo 5.4). Niveles plasmáticos elevados de TNF-α se asociaron a anorexia, malnutrición, anemia, caquexia muscular, hipertrigliceridemia, neuropatía uremica, hipertrofia ventricular izquierda y disfunción endotelial (resultados capitulo 5.5, 5.6.1, 5.7). En 1998, sugerimos la existencia de un grupo de complicaciones paralelas al estado de la uremia que se asociaban al TNF-α, proponiendo que el TNF-α

fuera incluido en la lista de tóxinas uremicas (resultados capitulo 5.5). Esto rompía la definición tradicional de toxina uremica propuesta por Bergström y Furst (44), ya que el TNF-α no se eliminaba por los métodos de diálisis, sino que por el contrario, se hiperproducia. Mas tarde, en el año 2000 otros autores definieron este síndrome con el nombre de “síndrome MIA, o malnutrición, inflamación arteriosclerosis” (29).

El MIA junta sabiamente la acumulación de citoquinas pro-inflamatorias que inducen caquexia y ateroesclerosis, explicando el elevado riesgo cardiovascular que presentan los pacientes en diálisis. En este sentido nos planteamos cómo afectaría la inflamación sistémica al primer evento que ocurre antes de iniciarse el proceso de ateroesclerosis. Es decir, su efecto sobre la disfunción endotelial. Fue así como encontramos que los pacientes que sufrían un MIA-2, presentaban una tendencia a la trombosis mediada por niveles bajos de tPA (el más poderoso fibronolitico) y altos de PAI (factor protrombotico), aumento en marcadores de daño endotelial como la trombomodulina (marcador de células endoteliales muertas) y del NO (vasodilatador) (resultados capitulo VI). En un seguimiento prospectivo (15 meses) de pacientes en diálisis que desarrollaron una inflamación sistémica demostramos dicha asociación causal (Capitulo 5.6.2).

Nuestros resultados y los de otros grupos con relación al síndrome malnutrición inflamación, nos hay llevado a plantear un ensayo clínico utilizando un anticuerpo monoclonal anti-TNF-α (infliximab) para tratar determinados pacientes en diálisis que sufren caquexia uremica. Este estudio debe completar el grupo de trabajos que durante estos años hemos desarrollado.

Pero no solo nos interesó el efecto deletéreo del TNF-α, si no los factores que condicionaban su hiperproducción. En este sentido encontramos que pacientes que padecían infecciones crónicas silentes como la infección por *Helicobacter pylori* (*HP*) sufrían anorexia y niveles plasmáticos más altos de TNF-α. Erradicamos el *HP* y los pacientes recuperaron al apetito, el estado nutricional y mejoraron la función endotelial (resultados capítulos 5.1 y 5.6.3). Esta mejoría se asoció a elevación de niveles plasmaticos de NPY y a descenso de los de TNF-α . Este hecho nos sugirió que otros procesos como prótesis vasculares y cualquier órgano enfermo e inflamado era potencialmente productor de TNF-α, como es el caso de pacientes con cardiopatías o hepatopatías. Sin embargo, en este mismo estudio encontramos un grupo de pacientes que sufrían anorexia y niveles de TNF-α similares al resto. Por ello investigamos la asociación entre anorexia y péptidos de diferente origen con acción sobre el ciclo hambre-saciedad. Uno de los más poderosos anorexigenos es la CCK, pero no encontramos diferencias en los pacientes estudiados (resultado capitulo 5.4). Otros anorexigenos como el péptido-C, GIP e IL-1 estaban elevados. Los orexigenos, el NPY y la ghrelina estaban relativamente bajos en pacientes anoréxicos. Finalmente, encontramos resultados variables en los niveles de ghrelina, leptina y NO medido como NO_3 (resultados capitulo 5.2 y 5.7).

Con relación al papel de la ghrelina en la modulación del apetito en pacientes en diálisis, investigamos los factores que influían en su liberación como la GH y la insulina. Nuestros resultados en este capitulo, y algunas observaciones previas (45), nos sugirieron que podria existir un trastorno en la liberación de la GH y que repercutiría en la liberación de ghrelina.

Efectivamente, encontramos una respuesta disminuida a la liberación de GH inducida por su hormona liberadora (GHRH), que fue experimentalmente estimulada por piridostigmina. Esto es indicativo de una atenuación de la regulación colinergica de la GH en el estado uremico (resultado capitulo 5.8).

En esta misma línea exploramos la influencia del metabolismo de la insulina, de péptidos gastrointestinales, de citoquinas pro-inflamatorias y adipocitoquinas derivadas de la masa grasa, sobre los trastornos en la conducta alimenticia en pacientes en diálisis. Encontramos que en general todos los pacientes presentaban curvas lentas de liberación de insulina, glucagón y péptido-C después de un estímulo alimenticio estándar. Con el mismo patrón, los pacientes anoréxicos, obesos, los uremicos sin trastornos del apetito y los controles presentaron curvas completamente distintas. Así mismo, la CCK, NPY, GIP, NO_3 y ghrelina, presentaron patrones distintos pero paralelos a sus respectivas curvas de insulina. La Leptina, TNF-α y la IL-6 participaron como orexigenos solo en fase basal. Sabemos que la insulina participa en la modulación de la liberación de estos péptidos, y que la insulina es modulada por la resistencia hidrocarbonada en células periféricas. Investigamos la expresión genética de leptina, TNF-α y adiponectina en muestras de grasa abdominal de pacientes a los que se les sometió a cirugías electivas, colocación o re-emplazo de catéteres de DP. Encontramos, una sobre-expresión de TNF-α en todos los uremicos, mas marcada en anoréxicos y en segundo lugar en obesos. Con relación a la leptina los uremicos presentaban una baja expresión mas marcada en obesos, lo que podría ser producto de los niveles sericos elevados de leptina. Una situación similar ocurrió con la adiponectina. Este patrón es el inductor de resistencia a la

insulina, la insulina regula muchos péptidos con acción sobre el apetito y por tanto podemos concluir que el tejido graso es un protagonista importante en la dis-regulación del apetito en el estado uremico (resultados capitulo 5.9).

Las alteraciones en la liberación de estos péptidos pueden ser también responsables de las preferencias y aversiones alimenticias que presentan los pacientes en diálisis. Es así como los niveles elevados de CCK o NPY producen saciedad preferentemente para hidratos de carbono (46, 47). Nosotros no encontramos asociación entre los niveles de estos péptidos y las preferencias alimenticias, pero la muestra seleccionada fue pequeña y nuestro objetivo en el trabajo (resultados capitulo 5.3) era valorar las preferencias alimenticias de nuestros pacientes con una encuesta validada para la población no renal. Esta es una area pendiente de estudio, ya que nuestros pacientes presentaron rechazo a carnes rojas, intermedio para carnes blancas y preferencia por hidratos de carbono y sabores intensos, posiblemente para neutralizar el sabor metálico o amargo asociado a la uremia.

Una de las últimas fases de nuestro estudio fue aplicar nuestros resultados a un eventual tratamiento para la anorexia y malnutrición en diálisis (33). En este sentido administramos acetato de megestrol 160 mg/día por tiempo variable a 32 pacientes en diálisis peritoneal. Encontramos una ganancia importante de peso al 3er mes y la albúmina plasmática aumentó al borde de la significación estadistica (resultado capitulo 5.10). Actualmente estamos analizando los resultados de un ensayo clínico a doble ciego, comparado con placebo. Uno de los resultados más interesantes es el aumento en sangre de NPY y descenso del TNF-α que se queda en el borde de la significancia estadística. Así mismo algunos resultados preliminares podrían

indicar que el acetato de megestrol aumenta la expresión genética de forma dosis dependiente de NPY en cultivo de una línea neuronal inmortal (anexo II). En este mismo sentido, tenemos en marcha un ensayo clínico utilizando infliximab como anti-TNF-α a pacientes malnutridos en diálisis. Hasta el momento se ha sido incluido un solo paciente. Lo mas relevante es que ha experimentado una elevación dramatica en los niveles de albúmina y sobre todo una mejoría muy significativa en la calidad de vida medida por el SF36.

Finalmente, y a medida que profundizamos en los mecanismos que controlan el apetito y sus alteraciones en el estado uremico, hemos propuesto la hipótesis del triptofano-serotonina (34), que conjunta todas las alteraciones conocidas encontradas por nosotros y otros grupos en una sola. Esta se basa en la elevada concentración del aminoácido triptofano en el liquido cefalo-raquídeo secundaria a una disminución en los BCCA secundaria a niveles elevados de TNF-α, CCK, leptina, ghrelina, insulina, acidosis, sobrecarga hídrica, deficiencia de NO-sintetaza (NOS) y de la enzima triptófano-hidroxilasa. Los niveles elevados de triptofano producen un síndrome hiperserotoninergico-like y como resultante anorexia severa y persistente (anexos I, fig. 1). Este trastorno no se corrige con diálisis y podría entrar dentro de la definición de la toxicidad de la uremia o bien llamarle síndrome metabólico-toxico de la uremia.

Aunque esta hipótesis no ha sido probada, ha recibido un importante soporte cientifico por la investigación de otros grupos (48-51). Demostrar esta hipótesis es parte de nuestro trabajo para próximos años. Además, investigaciones muy recientes indican que la ghrelina, un poderoso orexigeno

de muy reciente descubrimiento muestra un feed-back negativo con la serotonina cerebral (52).

7.- Conclusiones.

I.- Es necesario incorporar a la práctica clínica diaria una escala para medir el apetito en pacientes en diálisis como la escala analógica visual.

II.- La infección por *Helicobacter pylori* se asocia con anorexia, inflamación y malnutrición en pacientes en diálisis peritoneal. La erradicación del *HP* mejora significativamente este síndrome.

III.- La función renal residual tiene un efecto protector sobre la preservación de apetito, mas importante que la dosis de diálisis.

IV.- La conducta alimenticia en pacientes en DP es modulada por peptidos con acción central y periférica que estan elevados de forma basal y que se liberan anormalmente. Esto es debido a la retención renal de estos peptidos, a la interacción de los mismos entre sí, a la retención y sobreproducción de sustancias que normalmente se producen en pequeñas cantidades (toxinas urémicas) como el TNF-α, a desordenes en la liberación de hormona del crecimiento y a la intolerancia hidrocarbonada del urémico, que juega un papel clave en la liberación de los péptidos.

V.- La adipocitoquinas producidas por el tejido adiposo y la resistencia hidrocarbonada secundaria a estas sustancias tienen un papel predominante en la regulación del apetito en pacientes en diálisis.

VI.- Los niveles plasmáticos de leptina pueden ser considerados como marcador de riesgo cardiovascular y de ingesta de alimentos en pacientes no obesos sin inflamación.

VII.- La inflamación sistemica debido a la alta producción y acumulación de citoquinas proinflamatorias, inicia la disfunción endotelial y consecuentemente favorece procesos pro-trombóticos y pro-aterogénicos en pacientes en diálisis. Esto nos lleva a concluir lo importante que es erradicar infecciones crónicas silentes como el *helicobacter pylory*, accesos (prótesis de vasculares) infectadas. En general cualquier órgano enfermo es capaz de producir estas caquexinas.

VIII.- El acetato de megestrol mejora considerablemente el apetito y el estado nutricional posiblemente estimulando el NPY que es el más poderoso orexigeno conocido.

8.1.- Anexos I.

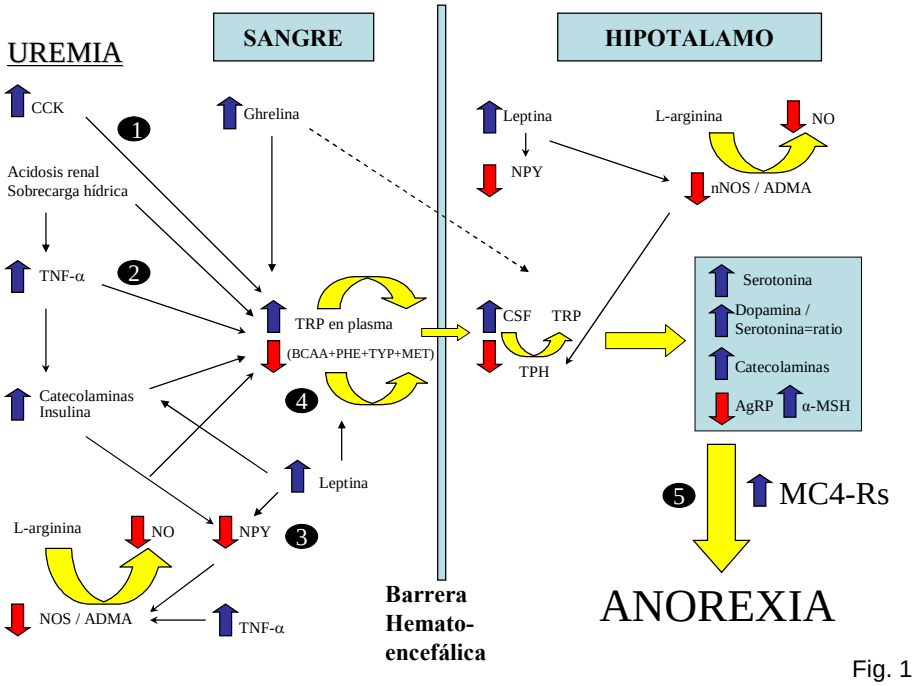

Fig. 1

Leyenda de la Figura 1. Hipótesis del triptofano serotonina. Patogénesis de la anorexia uremica y su tratamiento. CCK: colecistoquinina, IL-1: interleuquina-1. TNF-α: factor de necrosis tumoral alfa. TRP: triptofano. BCAA: aminoácidos ramificados de cadena corta. NO: óxido nítrico. NPY: neuropeptido Y. NOS: oxido nítrico sintetiza. nNOS: NO-sintetiza neural. ADMA: dimetil arginina L-asimétrica sintetaza. CSF: fluido cerebro espinal. TRH: triptofan hidroxilasa. αMSH: hormona estimulante de la melanocortina-alfa. AgRP: proteina relacionada a agouti. MC4-Rs: receptor 4 de la melanocortina. Tratamiento de la anorexia uremica los números significan el sitio especifico donde se puede actual desde el punto de vista terapéutico. (1): CCK antagonistas. (2): medicamentos anti-TNF-α (anticuerpos específicos, acetato de megestrol, talidomida, otros). (3): aumento del NPY. (4): ihnibidor del transporte del triptofano a través de la membrane hemato-encefálica (BCCA) (5): bloqueando el receptor de MC4-Rs.

8.2.- Anexos II.

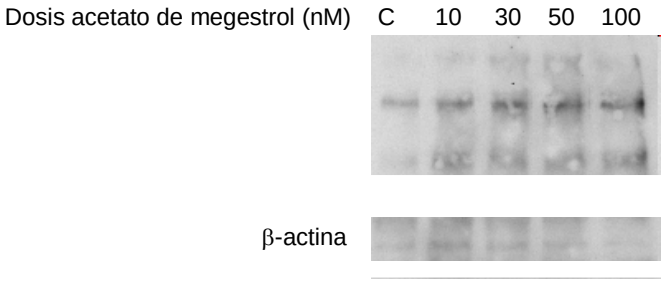

Fig. 2

Legenda de la figura 2. Wester blot. Las neuronas inmortales (SHSY.5Y), fueron lisadas (RIPA buffer). El extracto proteico fue guardado en congelación a -80 ºC. Para WB las proteínas fueron transferidas a una membrana de nitrocelulosa, que posteriormente fue bloqueada. Finalmente, se utilizó un anticuerpo primario y otro secundaria (anti-NPY humano). Para corregir la cantidad de proteína se utilizo la β-actina. El acetato de megestrol fue diluido en DMSO obteneiendo una concentración final major de 1 en 1 millon, los que no nos abligo a utilizar un grupo control con DMSO. La concentraciones molares del AM fuerón calculadas de acuerdo al peso molecular.

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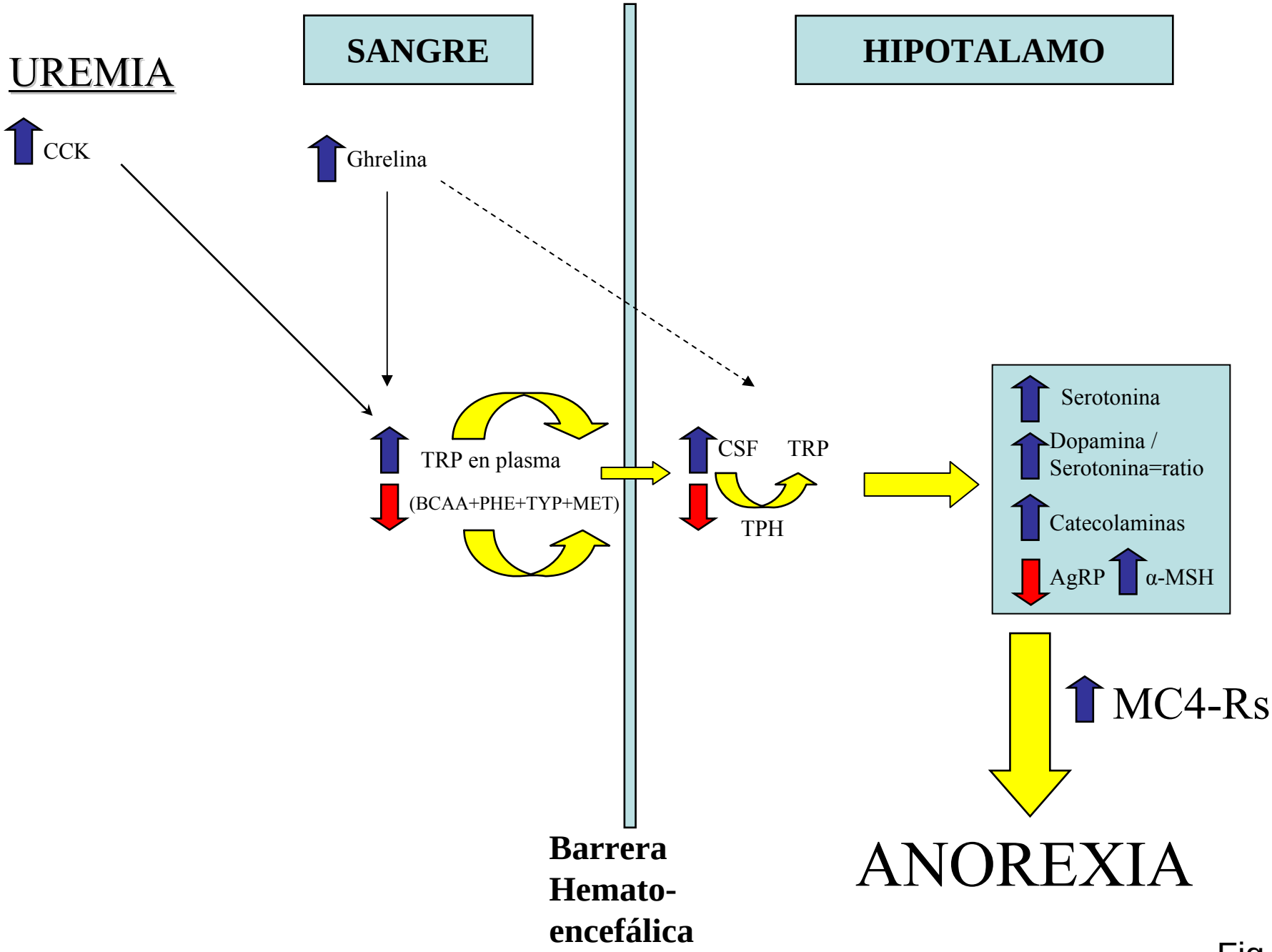
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Ghrelina
TRP en plasma
(BCAA+PHE+TYP+MET)
CSF
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Dopamina /
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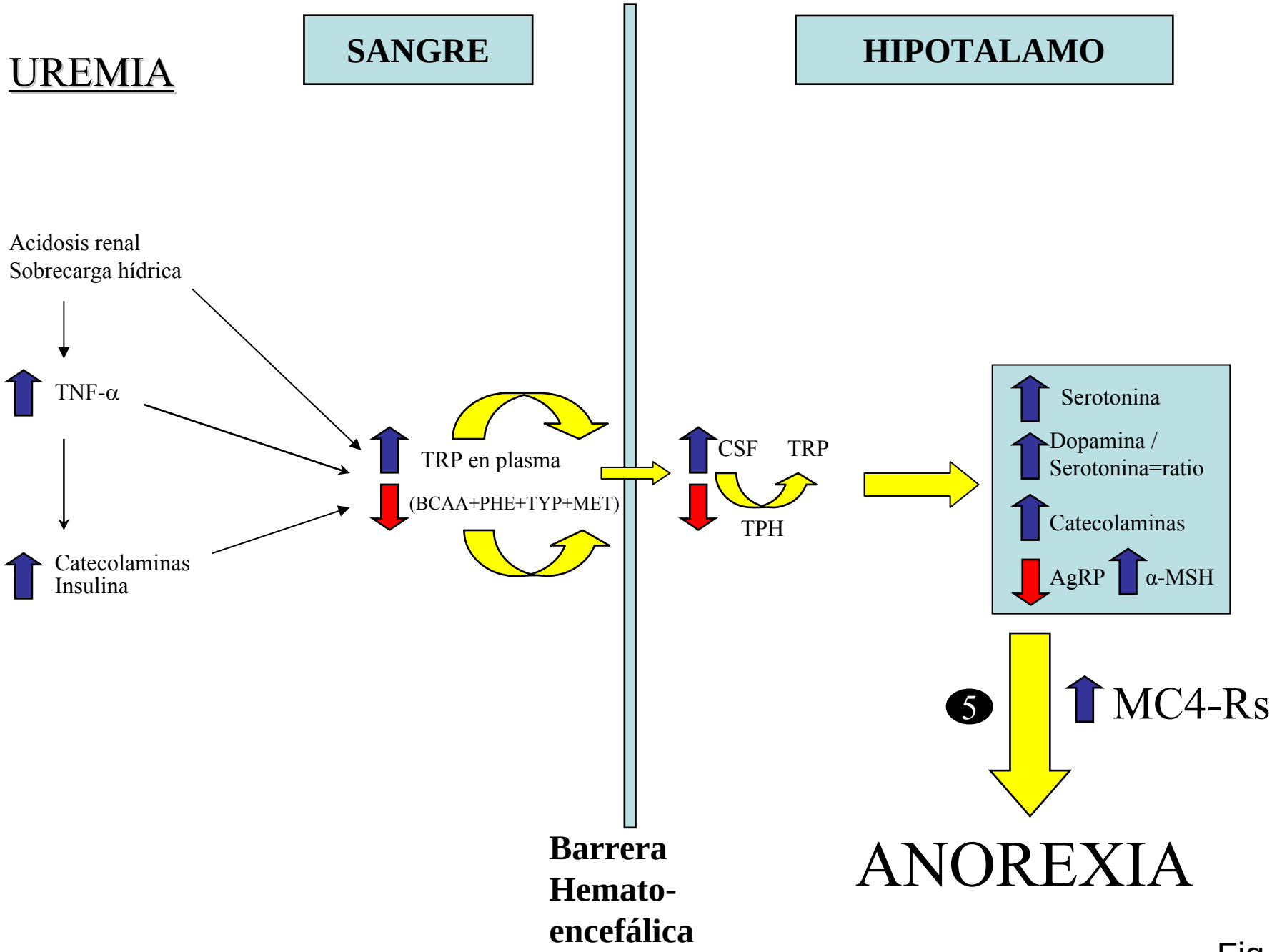
Fig. 1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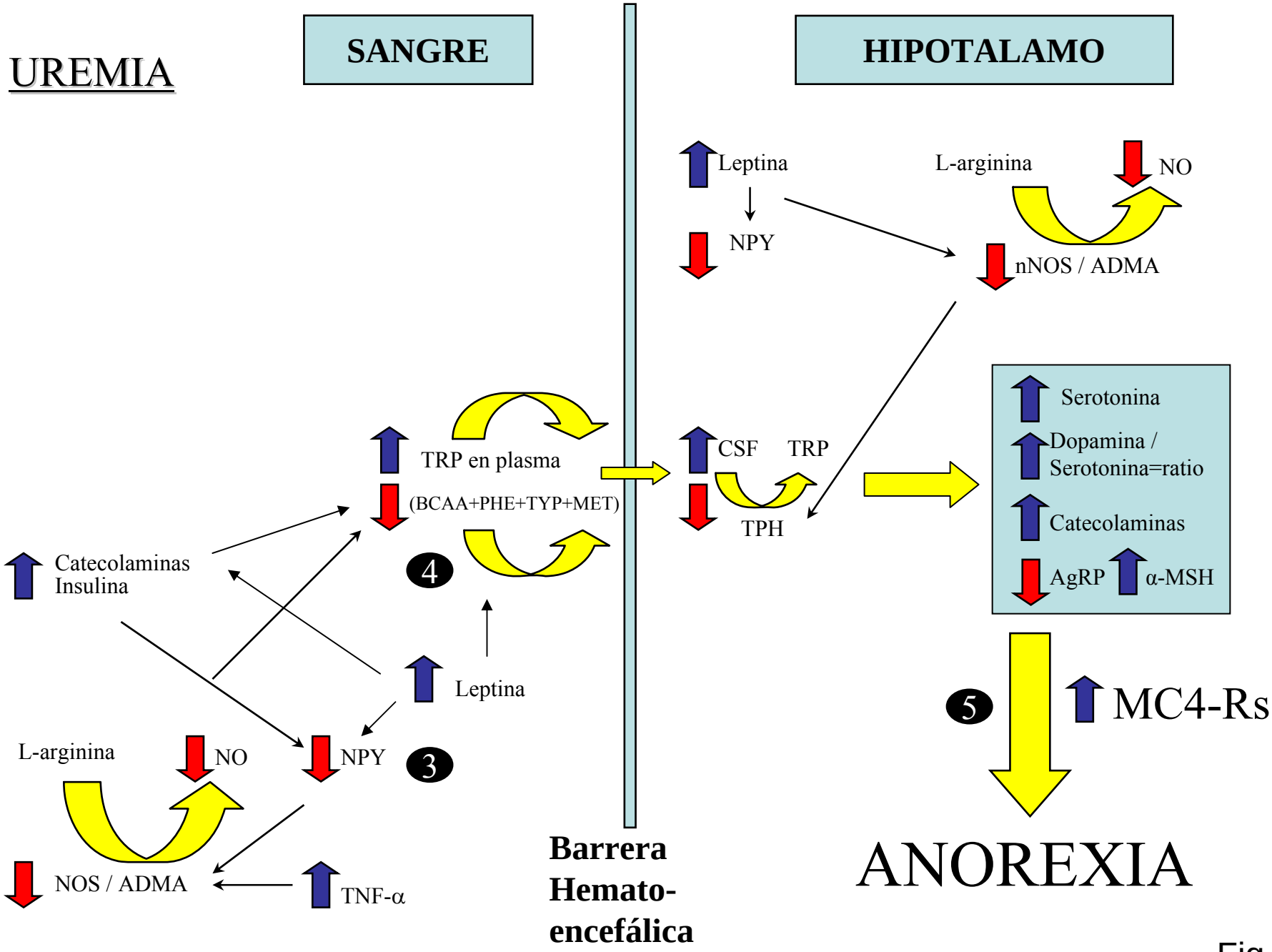
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Fig. 1

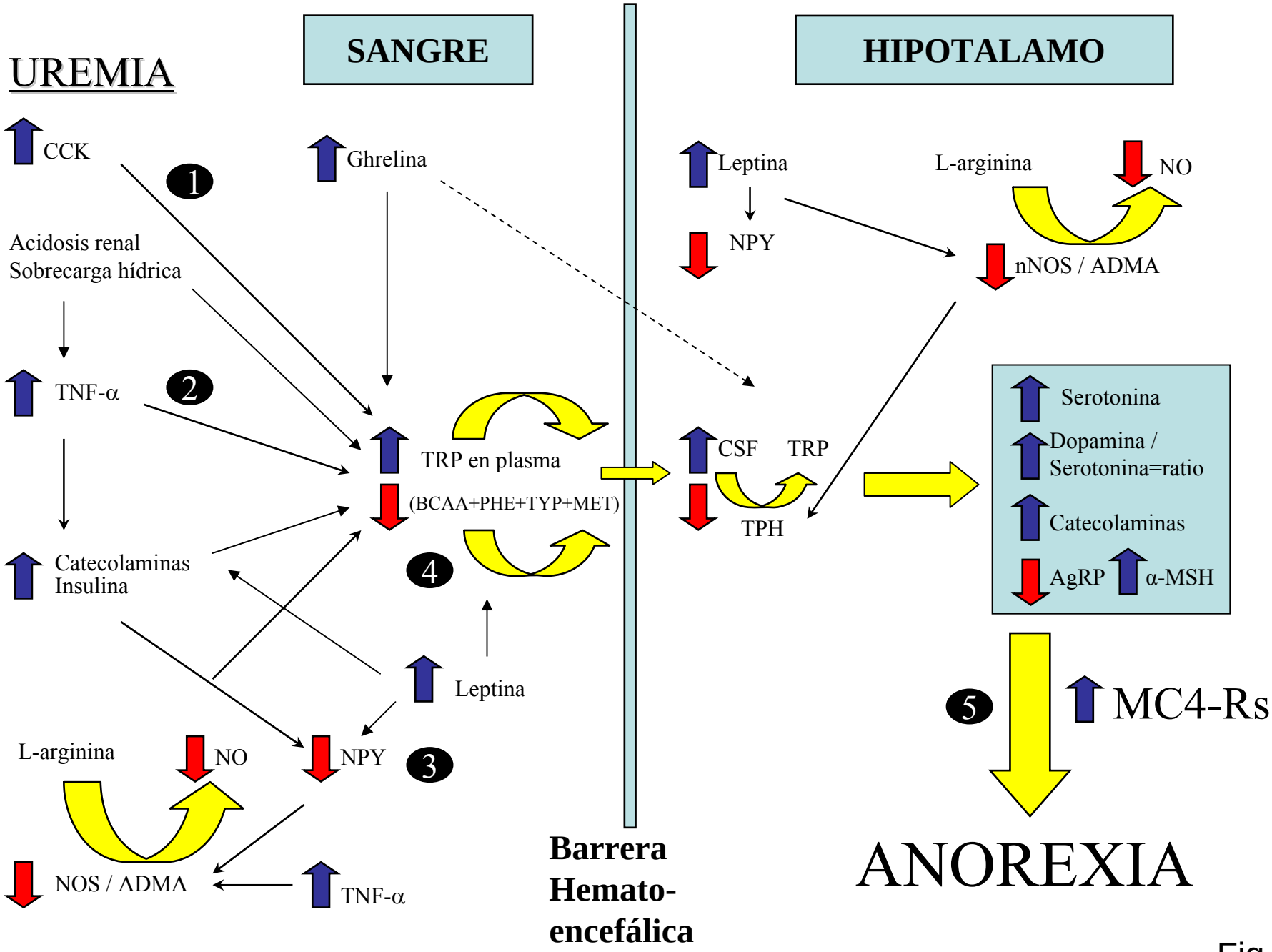
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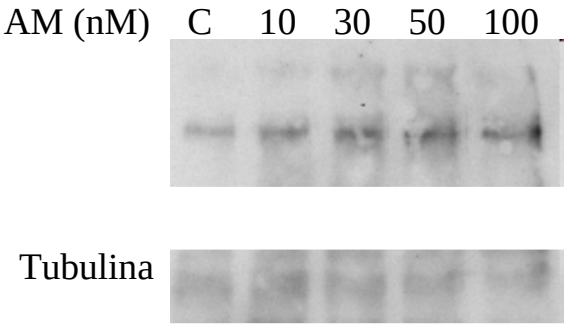
Fig. 1

Efecto del acetato de Megestrol sobre la expresión genética de NPY neuronas SHSY.5Y



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HELICOBACTER PYLORI INFECTION: A NEW CAUSE OF ANOREXIA IN PERITONEAL DIALYSIS PATIENTS

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♦ ***Objective: Helicobacter pylori (HP)*** **infection has frequently been found in dialysis patients. Chronic infections induce overproduction of pro-inflammatory substances. Inflammation has been associated with cachexia and anorexia. We explored the relationship between *HP* infection, anorexia, and malnutrition in peritoneal dialysis (PD) patients.**

♦ ***Patients and Methods:*** **The study included 48 clinically stable PD patients divided into four groups: *HP+* with anorexia (group I, $n = 12$); *HP+* without anorexia (group II, $n = 4$); *HP–* with anorexia (group III, $n = 5$); and *HP–* without anorexia (group IV, $n = 27$). Infection with *HP* was diagnosed by breath test. Anorexia was evaluated using a personal interview and an eating motivation scale (VAS). The VAS included five questions that are answered before and after eating. The questions concern desire to eat, hunger, feeling of fullness, prospective consumption, and palatability. Biochemical markers of nutrition and inflammation were also determined.**

♦ ***Results:*** **At baseline, group I showed lower scores for desire to eat, hunger sensation, prospective consumption, and palatability. They also showed lower lymphocyte counts, prealbumin, transferrin, serum albumin, normalized equivalent of protein–nitrogen appearance (nPNA), and residual renal function (RRF). In addition, the same group showed higher levels of C-reactive protein (CRP) and more sensation of fullness than the remaining groups. In the entire series, we found significant linear correlations between the following markers of nutrition and certain questions on the VAS: albumin with before-lunch desire to eat ($r = 0.38$, $p < 0.05$), and prealbumin with before-lunch hunger ($r = 0.41$, $p < 0.05$) and after-lunch hunger ($r = –0.35$, $p < 0.05$). Negative linear correlations were found between albumin and fullness before lunch ($r = –0.45$, $p < 0.01$), and between prealbumin and before-lunch desire to eat ($r = –0.39$, $p < 0.05$). Negative linear correlations were also seen between CRP and albumin ($r = –0.35$, $p < 0.05$) and between CRP and prealbumin ($r = –0.36$, $p < 0.05$). Similarly, CRP showed a negative correlation with before-lunch desire to eat ($r = –0.38$, $p < 0.05$) and after-lunch desire to eat ($r = –0.45$, $p < 0.01$). After *HP* eradication, group I showed a significant increase in markers of nutrition and in VAS scores for almost all questions. Simultaneously, they showed a decrease in CRP level. Significant differences were also found in lymphocyte count (1105 ± 259.4 cells/mm^3 vs 1330.8 ± 316 cells/mm^3, $p < 0.05$), nPNA (0.9 ± 0.16 g/kg/day vs 1.07 ± 0.3 g/kg/day, $p < 0.05$), prealbumin (26.7 ± 6.5 mg/dL vs 33.9 ± 56.6 mg/dL, $p < 0.01$), albumin (3.48 ± 0.3 g/dL vs 3.67 ± 0.35 g/dL, $p < 0.05$), CRP (1.16 ± 1.14 mg/dL vs 0.88 ± 1.2 mg/dL, $p < 0.054$), before-lunch desire to eat (56.6 ± 6.8 vs 72.2 ± 4, $p < 0.001$), after-lunch desire to eat (5.4 ± 2.6 vs 12.3 ± 2, $p < 0.01$), hunger before lunch (55.4 ± 5.4 vs 73.1 ± 4.6, $p < 0.001$), hunger after lunch (5.8 ± 2.9 vs 11 ± 4, $p < 0.01$), fullness before lunch (36.6 ± 10.3 vs 18.7 ± 8.8, $p < 0.001$), consumption after lunch (5 ± 4.7 vs 17.5 ± 18, $p < 0.05$), and palatability (61 ± 5.3 vs 74.1 ± 4.1, $p < 0.001$).**

♦ ***Conclusion:*** **Infection with *HP* is associated with anorexia, inflammation, and malnutrition in PD patients. Eradication of *HP* significantly improves this syndrome. Residual renal function seem to have a protective effect on appetite preservation. The present study supports the hypothesis of the involvement of inflammation in the pathogenesis of malnutrition in PD patients.**

KEY WORDS: Uremic anorexia; *Helicobacter pylori*; inflammation; malnutrition.

Anorexia is a symptom frequently associated with uremic toxicity. It is also a cause of malnutrition in dialysis patients. Malnutrition is associated with high morbidity and mortality in dialysis patients (1). Factors implicated in the pathogenesis of uremic anorexia include retention of substances with anorexigenic effects (satietins), a decrease in orexigenic substances, inflammation, and disorders in factors that usually modulate the hunger–satiety cycle—for example, amino acid levels and underdialysis, among others (2,3).

The role of inflammation has recently been emphasized as a key point in the genesis of uremic cachexia

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[hypothesis of malnutrition, inflammation, and atherosclerosis (MIA syndrome)] (4). One of the markers of the inflammatory process is C-reactive protein (CRP), which is associated with an increased risk of cardiovascular disease in healthy and uremic subjects. Increased levels of CRP appear to reflect the overgeneration of pro-inflammatory cytokines [interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis alpha (TNFα)] (4,5), all of which are able to inhibit appetite at the hypothalamus (6). In consequence, acute or chronic infections are recognized as being responsible for inflammation cycles that induce production of substances with anorectic and cachectic action (2).

Infection with *Helicobacter pylori (HP)* is a silent infectious process that has been related to anorexia in the normal population (7). Some authors have reported an increased prevalence of *HP* infection among dialysis patients (8). The hypothesis of the present study was that chronic infection by *HP* in uremic subjects may be a cause of anorexia. Eradicating the infection should lead to an improvement in appetite.

PATIENTS AND METHODS

We studied 48 peritoneal dialysis (PD) patients, 24 on continuous ambulatory peritoneal dialysis (CAPD) and 24 on APD (22 men and 26 women), ranging in age from 22 years to 79 years (mean: 56.9 ± 14 years). The mean time on PD was 17.5 ± 13.2 months (range: 3 – 63 months). No active acute or chronic disorders were present in the patients in the 3 months before the study. Patients with recognized endothelial disease (vasculitis, scleroderma, malignant hypertension), active systemic disease, or immunosuppression were excluded. The causes of renal failure in the study patients were glomerulonephritis (9 cases), diabetes (8 cases), chronic pyelonephritis (10 cases), polycystic kidney disease (6 cases), nephrosclerosis (10 cases), unknown etiology (4 cases), and other (1 case).

We assigned each patient to one of four groups according to the presence of anorexia and *HP* infection: *HP+* with anorexia (group I, $n = 12$); *HP+* without anorexia (group II, $n = 4$); *HP–* with anorexia (group III, $n = 5$); and *HP–* without anorexia (group IV, $n = 27$).

The study had two phases, including two analytical determinations and two breath tests: one at baseline and one after *HP* eradication in the positive cases. The therapeutic regime was omeprazole (40 mg daily), clarithromycin (1 g daily), amoxicillin (1 g daily), and clavulanic acid (250 mg daily) for 10 days.

DETERMINATION OF PARAMETERS

Infection with *HP* was diagnosed by breath test (Tau-Kit: Isomed SL, Madrid, Spain). This method is based on an oral intake of 100 mg of ^{13}C urea. In the presence of *HP*, urease hydrolyzes the ^{13}C urea, releasing $^{13}CO_2$, which is detected by mass spirometry in a breath sample. Patients in a fasting condition take half a glass of a citric solution (200 mL). After 10 minutes, the baseline breath sample (exhalation) is obtained. A solution of Tau-Kit plus water is immediately ingested, and 309 minutes later, the second breath sample is obtained. Diagnosis of *HP* infection by breath test is a recognized method with high sensitivity and specificity. It is currently the diagnostic method of choice (9).

Dialysis adequacy was assessed by weekly urea Kt/V and normalized equivalent of protein–nitrogen appearance (nPNA) (10).

For markers of nutrition, we measured serum albumin, transferrin, prealbumin, cholesterol, triglycerides, ferritin, and iron. Serum albumin was determined by a colorimetric method (Hitachi 704 analyzer: Hitachi, Madrid, Spain). Transferrin and prealbumin were measured by immunonephelometry (Boehringer nephelometer: Terminal SA, Madrid, Spain); cholesterol, by a colorimetric method; triglycerides, using the Hitachi 704; ferritin and iron, using the Hitachi 911 analyzer (Hitachi, Madrid, Spain).

Anorexia was evaluated by personal interview and an eating motivation scale [visual analog scale (VAS)] (11). The VAS included five questions to be answered before and after eating. The question dealt with desire to eat, hunger, feeling of fullness, prospective consumption, and palatability. Answers were given using a horizontal scale (0 – 100 mm). The scale score has no normal value; each subject is a personal control. However, a normal degree of before-lunch desire to eat generally ranges from 65 mm to 75 mm (11).

As a marker of inflammation, we measured CRP by immunonephelometry [ELISA Vectastain ABC kit: Vector Laboratories, Burlingame, CA, U.S.A. (normal range: <0.5 mg/dL)].

STATISTICAL ANALYSIS

Results are given as median and range. Comparisons between groups were performed using a nonparametric test, the Mann–Whitney rank-sum U-test. Spearman regression analysis and the Student t-test were used for paired and non paired data. A p value less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows the differences among the four groups at baseline. Patients from group I (*HP+* with anorexia) showed a lower lymphocyte count and lower prealbumin, transferrin, serum albumin, nPNA, and creatinine clearance than did the remain-

ing groups. The group I patients also showed higher CRP levels. These findings suggest a unique situation of the *HP*-infected patients with respect to the other groups.

Table 2 shows the differences in the VAS at baseline. Group I patients (*HP+* with anorexia) again showed significantly lower values in desire to eat, hunger, sensation of fullness, prospective consumption, and palatability—all indicating the poor state of their appetite.

In all patients studied ($n = 48$), we found significant linear correlations between the various markers of nutrition. Serum albumin correlated with prealbumin ($r = 0.43$, $p < 0.01$) and cholesterol ($r = 0.38$, $p < 0.05$); nPNA correlated with prealbumin ($r = 0.35$, $p < 0.05$). A linear correlation was also seen between markers of nutrition and VAS values: albumin with before-lunch desire to eat ($r = 0.38$, $p < 0.05$), and prealbumin with hunger before lunch ($r = 0.4$, $p < 0.05$) and hunger after lunch ($r = –0.35$, $p < 0.05$). A negative linear correlation appeared between albumin and fullness before lunch ($r = –0.45$, $p < 0.01$) and between prealbumin and before-lunch desire to eat ($r = –0.39$, $p < 0.05$).

A negative linear correlation was seen between CRP and albumin ($r = –0.35$, $p < 0.05$), prealbumin ($r = –0.36$, $p < 0.05$), before-lunch desire to eat ($r = –0.38$, $p < 0.05$), and after-lunch desire to eat ($r = –0.45$, $p < 0.01$).

Table 3 shows the changes in the markers of nutrition, CRP, and VAS scores in the infected patients with anorexia before and after *HP* eradication. A significant increase was observed in almost all markers of nutrition, together with a simultaneous decrease in CRP. The VAS parameters also showed significant and unequivocal improvement.

TABLE 1
Differences in General Parameters for the Four Groups at Baseline

Parameter	Group I	Group II	Group III	Group IV
Hemoglobin (g/dL)	11.4±0.9	11.8±0.5	10.4±1.4	11.4±0.9
Lymphocyte count (cells/mm^3)	1105±259[a,b]	1225±230	1350±62[a]	1525±184[b]
Prealbumin (mg/dL)	26.7±6.4[c,d]	32.7±9.9[c]	31.7±0.5	36.1± 2.5[d]
Transferrin (mg/dL)	177.6±27[f,g]	242±15[f]	210±47.7	233.4±35[g]
Cholesterol (mg/dL)	202±45	227±15.8	193±8.8	215±37
Serum albumin (g/dL)	3.4±0.25[h,i]	3.7±0.13	3.7±0.3[h]	3.9±0.15[i]
Serum creatinine (mg/dL)	7.7±2.3[j]	7.78±1.3	6.3±1.4	6±0.5[j]
nPNA (g/kg/day)	0.9±0.16[k]	1.03±0.1	0.89±0.14	1.08±0.1[k]
Weekly Kt/V	2±0.3[l]	2.1±0.19	2.1±0.24	2.21±0.11[l]
CRP (mg/dL)	1.16±1.14[m]	0.7±0.23	0.9±0.6	0.6±0.23[m]
C_{Cr} (mL/min)	2.7±2.3[n]	4.7±1.2	2.8±0.9	4.8±1.6[n]

nPNA = normalized equivalent of protein–nitrogen appearance; CRP = C-reactive protein; C_{Cr} = creatinine clearance.
[a – n] Groups in which the differences were statistically significant ($p < 0.05$).

TABLE 2
Differences in Eating Motivation Between the Groups at Baseline Using a Visual Analog Scale (mm)

Parameters	Group I	Group II	Group III	Group IV
Desire to eat				
Before lunch	56.6±6.8[a,b]	74±4.1[a]	61.2±4.5	74.5±6[b]
After lunch	5.4±2.6[c]	7±2.7	7.5±2.9	9±4[c]
Hunger				
Before lunch	55.4±5.4[d,e]	72±4[d]	61±2.5	73.7±5[e]
After lunch	5.8±2.9[f]	10±6	8.7±2.5[f]	8±4.8
Fullness				
Before lunch	36.6±10.3[g]	15±5[g]	32.5±3	14.7±6
After lunch	85±26	73±4.4	91.2±8.5	80.3±21.5
Prospective consumption				
Before lunch	57.9±5[h]	72±5.7[h]	61.2±4.8	70±5.9
After lunch	5±4.7[i,j]	13±6.7[i]	5±5.7	10.7±3.9[j]
Palatability	61±5.3[k,l]	75±3.5[k]	61±12.5	74.9±4[l]

[a – l] Groups in which the differences were statistically significant ($p < 0.05$).

TABLE 3
Changes in *Helicobacter pylori (HP)*–Infected Patients After *HP* Eradication

Parameters	Pre-treatment	Post-treatment	*p* Value
Lymphocyte count (cells/mm^3)	1105±259.4	1330.8±316	<0.05
nPNA (g/kg/day)	0.9±0.16	1.07±0.3	<0.05
Prealbumin (mg/dL)	26.7±6.5	33.9±56.6	<0.01
Albumin (g/dL)	3.48±0.3	3.67±0.35	<0.05
CRP (mg/dL)	1.16±1.14	0.88±1.2	=0.054
Desire to eat			
Before lunch	56.6±6.8	72.2±4	<0.001
After lunch	5.4±2.6	12.3±2	<0.01
Hunger			
Before lunch	55.4±5.4	73.1±4.6	<0.001
After lunch	5.8±2.9	11±4	<0.01
Fullness			
Before lunch	36.6±10.3	18.7±8.8	<0.001
After lunch	85±26	73±14.6	NS
Prospective consumption			
Before lunch	57.9±5	66±18	NS
After lunch	5±4.7	17.5±18	<0.05
Palatability	61±5.3	74.1±4.1	<0.001

nPNA = normalized equivalent of protein–nitrogen appearance; CRP = C-reactive protein.

The four patients from group II (*HP+* without anorexia) showed no changes in their markers of nutrition. The values before and after *HP* eradication were these: lymphocyte count [1225.8 ± 230.7 cells/mm^3 vs 1381 ± 297.8 cells/mm^3, nonsignificant (NS)], prealbumin (32.9 ± 10 mg/dL vs 33.8 ± 5.6 mg/dL, NS), transferrin (242 ± 15 mg/dL vs 256.8 ± 19.6 mg/dL, NS), albumin (3.7 ± 0.13 g/dL vs 3.9 ± 0.3 g/dL, $p < 0.01$), before-lunch desire to eat (74 ± 4.1 vs 75 ± 5, NS) and after-lunch desire to eat (7 ± 2.7 vs 6 ± 3.1, NS), hunger before lunch (72 ± 4.4 vs 73 ± 2.7, NS) and hunger after lunch (10 ± 6.1 vs 8 ± 2.7, NS), fullness before lunch (15 ± 5 vs 20 ± 3, NS) and fullness after lunch (10 ± 6.1 vs 8 ± 2.7, NS), prospective consumption before lunch (72 ± 5.7 vs 73 ± 5, NS) and prospective consumption after lunch (13 ± 6.7 vs 11 ± 4.2, NS), and palatability (75 ± 3.5 vs 74 ± 2.2, NS). Levels of CRP showed a nonsignificant decrease (0.7 ± 0.23 mg/dL vs 0.6 ± 0.3 mg/dL, NS).

Because groups III and IV (both *HP–*) underwent no medical intervention, the results of their second analysis are not shown.

DISCUSSION

The most important finding of the present study is that it provides the first clinical support for the hypothesis linking inflammation and malnutrition (via anorexia) in PD patients suffering a chronic, symptomatic (anorexia), but silent infection caused by *Helicobacter pylori.* The distinct behavior differences between the two infected groups suggests that the presence of anorexia in this infection is quite relevant. Only those patients with coexisting anorexia and *HP* infection showed significant changes in appetite and nutrition after eradication of the infection (Table 3).

We used three approaches to evaluate anorexia, inflammation, and malnutrition. The VAS (11) appeared to be a good instrument for dialysis patients with appetite disorders. In the present study, it was able to establish differences in diverse situations of anorexia. The correlation between the VAS data and the markers of nutrition supports the VAS measurements.

The results shown in Table 2 reveal important differences in eating behavior. Patients with existing anorexia who were also infected with *HP* (group I) showed lower desire, hunger, prospective consumption, and palatability than did the other groups. The inverse relationships between CRP, VAS, and markers of nutrition also support the link between anorexia and inflammation.

The pathogenesis of uremic anorexia nevertheless appears to be more complex than being caused exclusively by inflammation. In the present study, a group of patients with no inflammatory signs (group III) suffered anorexia. In this regard, we recently published a hypothesis concerning other anorexia-inducing mechanisms, such as gastrointestinal hormones (3).

Residual renal function (RRF) has been considered to protect dialysis patients from morbidity and mortality (12). In this sense, RRF appears more important than weekly peritoneal urea Kt/V in appetite

preservation among our patients (Table I). Group IV, in effect, showed the best health conditions (no inflammation, best nutrition status, and highest RRF).

The important contribution of RRF has been suggested previously by others (12,13). Residual renal function may protect infected patients from cytokine accumulation; patients with no RRF may suffer the effects of similar cytokine production more intensively. This hypothesis concurs with the recognized sensitivity of uremic patients to infectious insult, in terms of general well-being, appetite, and food intake. The renal inability to eliminate cytokine excess would prolong the effects of the insult on heart, gastrointestinal tract, liver, and blood vessels through the accumulated mediators (14–16). The fact that *HP* infection has been associated with hyperproduction of cytokines in non uremic patients (17,18) supports that idea.

Our findings point to the importance of diagnosis and early treatment of *HP* infection in dialysis patients, especially when they show anorexia and signs of malnutrition. Treatment of *HP* infection after an appropriate diagnosis should precede other approaches such as anabolics, orexigen drugs, or dietary supplements.

CONCLUSION

Infection with *HP* is associated with anorexia, inflammation, and malnutrition in PD patients. Eradication of the *HP* infection significantly improves the entire syndrome. Residual renal function may have an overarching role in appetite preservation, especially in infected patients. Our findings give concrete support to the hypothesis of inflammation as a cause of malnutrition in dialysis patients.

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Ghrelin Plasma Levels and Appetite in Peritoneal Dialysis Patients

Abelardo Aguilera, Antonio Cirugeda, Ruth Amair, Gabriela Sansone, Laura Alegre, Rosa Codoceo, M. Auxiliadora Bajo, Gloria del Peso, Juan J. Díez, José A. Sánchez–Tomero, Rafael Selgas

*Anorexia-associated malnutrition is a severe complication that increases mortality in peritoneal dialysis (PD) patients. Ghrelin is a recently-discovered orexigenic hormone with actions in brain and stomach. We analyzed, in 42 PD patients, the possible relationship between ghrelin and appetite regulation with regard to other orexigens [neuropeptide Y (NPY), NO_3] and anorexigens [cholecystokinin (CCK), leptin, glucose-dependent insulinotropic peptide (GIP), tumor necrosis factor alpha (TNFα)]. All orexigens and anorexigens were determined in plasma. Eating motivation was evaluated using a visual analog scale (VAS). The patients were divided into three groups: those with anorexia (*n *= 12), those with obesity associated with high intake (*n *= 12), and those with no eating behavior disorders (*n *= 18). A control group of 10 healthy volunteers was also evaluated.*

*Mean plasma levels of ghrelin were high (3618.6 ± 1533 pg/mL), with 36 patients showing values above the normal range (<2600 pg/mL). Patients with anorexia had lower ghrelin and NPY levels and higher peptide-C, CCK, interleukin-1 (IL-1), TNFα, and GIP levels than did the other patients. Patients with anorexia also had an early satiety score and low desire and pleasure in eating on the VAS and diet survey. We observed significant positive linear correlations between ghrelin and albumin (*r *= 0.43,* p *< 0.05), prealbumin (*r *= 0.51,* p *< 0.05), transferrin (*r *= 0.4,* p *< 0.05), growth hormone (*r *= 0.66,* p *< 0.01), NO_3 (*r *= 0.36,* p *< 0.05), and eating motivation (VAS). At the same time, negative relationships were observed between blood ghrelin and GIP (*r *= –0.42,* p *< 0.05), insulin (*r *= –0.4,* p *< 0.05), leptin (*r *= –0.45,* p *< 0.05), and creatinine clearance [*r *= –0.33,* p *= 0.08 (nonsignificant)]. Ghrelin levels were not related to Kt/V or to levels of CCK and cytokines.*

Ghrelin plasma levels are elevated in PD patients. Uremic patients with anorexia show relatively lower ghrelin plasma levels than the levels seen in obese patients or in patients with normal appetite. The role of ghrelin in appetite modulation is altered in uremic PD patients, and that alteration is possibly associated with disorders in insulin and growth hormone metabolism.

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Key words
Eating behavior disorders, malnutrition, inflammation, ghrelin

Introduction

The deleterious effects of malnutrition in dialysis patients are well recognized. Malnutrition is a common factor in the two major causes of death during dialysis: cardiovascular disease and infection (1). Anorexia is frequently associated with uremia and represents the first step in malnutrition (2). Uremic status complicates appetite regulation because it provokes disorders in adipose tissue, the gastrointestinal system, neuropeptide protection and retention, inflammation, and the central nervous system. We have suggested the possibility that, in uremic patients, a true imbalance exists between anorexigenic and orexigenic mediators (3)—a misbalance that favors the appearance of anorexia.

Ghrelin is a novel 28-amino-acid octanoylated peptide that has been identified in stomach as an endogenous ligand for the growth hormone (GH) secretagogue receptor. Ghrelin is a powerful orexigen in fasting conditions; and, because its postprandial cycle is inverse to the glucose and insulin curves, it has satiating properties. Ghrelin influences eating consumption by increasing the desired meal size, and the peptide has been associated with bulimia–anorexia in non uremic patients (4). Recently, it has been suggested that renal retention may significantly elevate ghrelin plasma levels in patients with renal failure (5). Our hy-

pothesis derives from the contradiction between elevated ghrelin levels and the strong tendency to anorexia in uremic patients. In the present study, we analyzed the relationship between ghrelin plasma levels, other appetite regulators, and markers of nutrition in peritoneal dialysis (PD) patients.

Patients and methods

We studied 42 clinically stable PD patients [20 on continuous ambulatory PD (CAPD), 22 on automated PD (APD)]. The 22 male and 26 female patients ranged in age from 22 years to 79 years (mean: 56.9 ± 14 years). Their mean period on PD was 17.5 ± 13.2 months (range: 3 – 63 months). Causes of renal failure were nephrosclerosis (10 cases), glomerulonephritis (8 cases), diabetes (7 cases), chronic pyelonephritis (6 cases), polycystic kidney disease (5 cases), unknown (4 cases), and others (2 cases).

We used a visual analog scale (VAS) adapted from Hill and Blundell [see (6)] to evaluate eating motivation. The VAS includes 5 questions about desire, hunger, sense of fullness, prospective consumption, and palatability to be answered before and after eating. Answers are marked on a horizontal scale (0 – 100 mm).

Anorexia was defined as low eating motivation (personal interview and VAS < 60 mm), low food intake [normalized protein equivalent of nitrogen appearance (nPNA) < 1.1 g/kg/day, daily dietary assessment < 30 kcal/kg], and low values for markers of nutrition [Dialysis Outcomes Quality Initiative clinical practice guideline for nutrition (7)].

Obesity with high food intake was considered when the patient's body mass index (BMI) measured 25 – 30 kg/m^2 [grade I, World Health Organization (WHO) criteria (8)], 30 – 40 kg/m^2 (WHO grade II), or >40 kg/m^2 (WHO grade III); when eating motivation was high (VAS > 60 mm); and when daily food intake (6) or bulimic criteria [*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (9)] was high.

Normal eating behavior was considered in the absence of anorexia (VAS) and bulimia, with a normal BMI (18.5 – 25 kg/m^2) and normal values for markers of nutrition (7).

Using those definitions, we divided the study patients into 3 groups: those with anorexia (n = 12), those with obesity (n = 12) and high food intake, and those without eating behavior disorders (n = 18). We also studied a control group of 10 healthy volunteers (6 men, 4 women).

For all patients and controls, we determined these parameters:

- Dialysis adequacy:
  - Kt/V urea and nPNA
  - Markers of nutrition:
  - Long-term: plasma creatinine, albumin, cholesterol (colorimetric method, Hitachi 704: Boehringer Mannheim, Mannheim, Germany), and transferrin (immunonephelometry, Behring Nephelometer: Behringwerte AG, Marbus, Germany)
  - Medium-term: short, half-life plasma proteins such as prealbumin and retinoid-binding protein [RBP (immunonephelometry, Behring Nephelometer)], serum GH [immunoenzymatic assay, AIA 1200: Tosoh Corporation, Tokyo, Japan; maximum intra-assay and inter-assay coefficients of variation, 5.4% and 3.3% respectively; sensitivity, 0.1 ng/mL (normal, <5 ng/mL)]
  - Short-term: urea nitrogen, serum phosphate, and serum potassium. Mean daily dietary intake was determined from individual 24-hour food records during a 3-day period (including 1 weekend day). Daily calories and intake of carbohydrates, lipids, and protein were calculated for each patient using commercially available computer software [Wander (1990): Sandoz Nutrición, Barcelona, Spain].
- Plasma or serum peptide appetite modulators (radioimmunoassay):
  - Glucose (hexoquinase reaction: Boehringer Mannheim; normal fasting levels, 90 – 120 mg/dL)
  - Insulin (Sorin: Biomedica, Saluggia, Italy; sensitivity, 3 mIU/mL; intra-assay and inter-assay coefficients of variation, 6.6% and 6.2% respectively)
  - Glucose-dependent insulinotropic peptide [GIP (Peninsula Laboratories, Belmont, CA, U.S.A.); normal range, 35 – 52 pg/mL]
  - Cholecystokinin [CCK, 26–33 unsulfated fragment (Peninsula Laboratories); normal value, 12 – 20 pg/mL], which shows anorexigenic action

- Leptin (Linco Research, St. Louis, MO, U.S.A.; sensitivity, 0.5 ng/mL; linearity, 100 µg/L; normal range, 3 – 7.8 ng/mL), which has an anorexigenic effect
- Neuropeptide Y [NPY (Peninsula Laboratories); normal range, 220 – 370 pg/mL], a powerful orexigen
- Ghrelin (RIA, 125-Ighrelin: Linco Research; sensitivity, 100 pg/mL; normal range in our 10 healthy volunteers, 900 – 2500 pg/mL), which shows anorexigenic action
- NO [measured as serum NO_3, a final metabolite of NO, by capillary electrophoresis; normal range, 90 – 110 µmol/L (10)], a powerful orexigen
- Cytokines with recognized action on the hunger–satiety cycle: tumor necrosis factor alpha [TNFα (ELISA, Medigenix Easia kit: Biosource Europe SA, Nivelles, Belgium); normal, 3 – 20 pg/mL] and interleukin-1 [IL-1 (ELISA, Medigenix Easia kit); normal, <15 pg/mL]

Statistical analysis

Results are given as medians and ranges. Comparisons between study groups were performed using a nonparametric test, the Mann–Whitney rank sum *U*-test. Spearman regression analysis and the Student *t*-test were used for paired and unpaired data. A *p* value less than 0.05 was considered statistically significant.

Results

Table I shows the demographic and hematologic characteristics of the patients at baseline. Patients with anorexia were older and showed lower nPNA, albumin, prealbumin, RBP, and daily food intake. They also showed higher plasma levels of TNFα and IL-1 than were seen in the other groups. Relatively higher plasma levels of anorexigens (CCK, TNFα) and lower levels of orexigenic substances (NPY, ghrelin) were present in the group with anorexia. The contrary was observed in the obese group. Table I also shows a clear difference in ghrelin plasma levels between the dialysis patients and the healthy controls.

Table II shows the data from the VAS. Before and after eating, patients with anorexia scored differently than the other patients, confirming poor appetite in the anorectic group. Patients with obesity presented the opposite eating attitude.

Table III shows significant linear correlations between VAS scores and plasma levels of CCK, NPY, ghrelin, leptin, TNFα, and NO_3. Those results effectively confirm the associations between anorexia and CCK, leptin, and TNFα. On the other hand, NPY, ghrelin, and NO_3 were associated with higher eating desire.

In PD patients, ghrelin plasma levels were 3618.6 ± 1533 pg/mL as compared with 2084 ± 533.3 pg/mL in healthy controls, $p < 0.01$. Most patients ($n = 36$) showed values above the normal range.

We found significant positive linear correlations between ghrelin and some nutritional markers: albumin ($r = 0.43$, $p < 0.05$), prealbumin ($r = 0.51$, $p < 0.05$), transferrin ($r = 0.4$, $p < 0.05$), GH ($r = 0.66$, $p < 0.01$), and NO_3 ($r = 0.36$, $p < 0.05$). Ghrelin also showed negative correlations with GIP ($r = –0.42$, $p < 0.05$), insulin ($r = –0.4$, $p < 0.05$), leptin ($r = –0.45$, $p < 0.05$), and creatinine clearance ($r = –0.33$, $p = 0.08$, NS). Ghrelin was not significantly related to Kt/V urea, CCK, or cytokine levels.

Levels of NPY showed a negative linear correlation with IL-1 ($r = –0.52$, $p < 0.05$) and TNFα ($r = –0.51$, $p < 0.05$), and TNFα and IL-1 showed a positive linear correlation ($r = 0.85$, $p < 0.005$). Levels of IL-1 and CCK also showed a positive linear correlation ($r = 0.45$, $p < 0.05$), as did levels of IL-1 and GIP ($r = 0.46$, $p < 0.05$).

Discussion

Eating behavior is a complex phenomenon with sociocultural and biologic influences, and eating is complicated by the resulting profound metabolic alterations and retention of catabolic end-products (3,4).

Dialysis patients have a strong tendency toward anorexia. They retain substances with anorectic action such as CCK, leptin, corticotropin-releasing hormone, insulin, glucagons, TNFα, IL-1, GIP, lack of NO, C-peptide, α-melanocyte-stimulating hormone (α-MSH), and free tryptophan (11). All of those agents promote the transfer of high concentrations of free tryptophan into cells—most especially in the hypothalamus—resulting in increased serotonin production and loss of appetite [tryptophan–serotonin hypothesis (12)].

Ghrelin, a novel 28-amino-acid polypeptide with GH secretagogue action, increases food intake, fat accumulation, body weight, gastric acid secretion, and stomach motility; it also reduces blood pressure, im-

TABLE I Demographics and biochemical and nutrition markers in the study groups

	Patients				
	Anorexic (A)	*Obese (O)*	*Normal appetite (N)*	*Controls (C)*	p *Value*
Age (years)	66.4±10	56.3±7.1	49.7±14	31±3.7	A vs. N, <0.05 O vs. C, <0.05 N vs. C,<0.05 A vs. C, <0.001
PD duration (months)	36.8±32.3	23±11.5	45.5±46.7	—	NS
CCr (mL/min)	0.5±0.45	1.42±1.01	1.38±1.39	101±7	A/O/N vs. C, <0.001
Daily nPNA (g/kg)	0.87±0.21	1.1±0.25	1.14±0.11		A vs. N, <0.05
Weekly Kt/V urea	2±0.25	1.98±0.33	2.17±0.33		NS
Cholesterol (mg/dL)	174±57.4	211±55.6	188±56	184±30	NS
Albumin (g/dL)	3.7±0.08	4±0.2	3.9±0.4	5±0.4	A vs. O/C, <0.05
Transferrin (mg/dL)	209±36	262±47	205±50.7	303±57.2	NS
Prealbumin (mg/dL)	26±7	31±2.9	31±7.5	34±3	A vs. O/C, <0.05
Retinol protein binding (mg/dL)	8.4±3	11.5±3	13±2	5.3±1.2	A vs. O/N, <0.05
Lymphocytes/mm^3	1298±444	1452±613	1727±480	1877±592	NS
Growth hormone (ng/mL)	3.4±3.8	4±4.8	2.2±1.4	1.7±1.7	NS
Diet survey (kcal/day)	1277±467.4	2320±179.4	2006±351	2089±339	A vs. O, <0.01 A vs. N/C, <0.05
Fat (kcal/day)	60.4±28.9	102±23.2	98±22	74.7±15	A vs. O/N, <0.05 O vs. C, <0.05
Protein (kcal/day)	63±18	85.7±16.6	83.8±13.7	74.5±21.8	A vs. O, <0.05
Carbohydrates (kcal/day)	98±41	227±71	155.5±27	248.8±67.2	A vs. O/N/C, <0.01
Glucose (mg/dL)	93±33	101±40	105±69	81±5	A vs. N/C, <0.05 O vs. C, <0.05
Insulin (mIU/mL)	34±24	41.3±50.7	13.8±4.9	12.4±3.2	A vs. N/C, <0.05 O vs. C, <0.05
GIP (pg/mL)	101.2±22	111.8±28	132.6±25	47.1±7.3	A/O/N vs. C, <0.01
Cholecystokinin (pg/mL)	25.8±3.7	19.9±4.1	21.6±8	10.9±1.8	A vs. O, <0.05 N vs. C, <0.05
Leptin (ng/mL)	44.5±45	110±45	35±28	11.8±8	A vs. O, <0.01 O vs. N, <0.01 N vs. C, <0.01
Neuropeptide Y (pg/mL)	369±260	463.5±61.6	433.7±61	320.5±48	A vs. O/N/C, <0.05
Ghrelin (pg/mL)	3646±958.6	4486±1614.8	4289±1161	2084±533.3	A/O/N vs. C, <0.05
NO_3 (μmol/L)	175±60	190±35.9	152±26.6	92.7±7.5	A vs. C, <0.001 N vs. C, <0.001 O vs. C, <0.05
TNFα (pg/mL)	121±43.8	40±11.6	38.2±16	18±4	A vs. O/N, <0.01
IL-1 (pg/mL)	6.12±0.8	2.1±0.43	2.2±1.34	1±0.8	A vs. O/N, <0.001

PD = peritoneal dialysis; NS = nonsignificant; CCr = creatinine clearance; nPNA = normalized equivalent of protein nitrogen appearance; GIP = glucose-dependent insulinotropic peptide; TNFα = tumor necrosis alpha; IL-1 = interleukin-1.

proving cardiac function (5,13). The high ghrelin plasma levels we found in PD patients correlate negatively with residual renal function (RRF), indicating that RRF is responsible at least in part for ghrelin accumulation. Our findings also confirm findings by Yoshimoto A *et al.* (5).

Importantly, dialysis dose (Kt/V) does not seem to play an important role in uremic ghrelin accumulation, supporting the idea that RRF is more important than dialysis dose in preserving appetite in uremia (11,14). We found a close association between fasting ghrelin plasma levels and eating motivation as measured by VAS (Table III). We also found a positive relationship between fasting ghrelin plasma levels and markers of nutrition. Moreover, patients with anorexia showed relatively lower values of ghrelin than did obese patients or patients with normal appetite (Table I). The anabolic effect of ghrelin is the result of its stimulation

TABLE II Motivation to eat, measured by the visual analog scale [a]

	Patients				
	Anorexic (A)	*Obese (O)*	*Normal appetite (N)*	*Controls (C)*	p *Value*
Desire to eat before lunch	60±6.1	76.6±6	67.8±6.9	72.8±3.9	A vs. O/C, <0.01
Desire to eat after lunch	8.6±2.2	21.6±4	13.2±5	13.5±8.5	A vs. O, <0.05
Hunger before lunch	60±6.1	78.3±6	68.6±4.7	74.3±4.5	A vs. O/N, <0.001 A vs. C, <0.01 O vs. N, <0.01
Hunger before lunch	8±4.4	21.6±4	12.8±5.5	17.1±4.8	A vs. O/C, <0.01
Fullness before lunch	28±8.4	18.8±2.5	12.5±4.2	11.8±4.1	A vs. N/C, <0.01
Fullness after lunch	81±5.4	59.1±19.6	77±5.6	77±5.6	A vs. O, <0.05 O vs. C, <0.05
Prospective consumption before lunch	59±5.5	78.3±4	71.4±3.7	75.7±4.5	A vs. O/N/C, <0.001 O vs. N, <0.01
Prospective consumption before lunch	6±2.2	25±5.4	12.3±2.7	13.5±4.7	A vs. O, <0.001 A vs. N/C, <0.01 O vs. N, <0.01
Palatability	60±7	75±5.4	71.4±4.7	74.3±5.3	A vs. O/N/C, <0.01
Hunger 2 hours before lunch	34±5.4	58.3±2.6	45±8.6	45.7±5.3	A vs. O/C, <0.01 A vs. N, < 0.05 O vs. N/C, <0.01
Satiety 2 hours after lunch	60±0	39. 2±6.6	40.7±17.4	40±0	A vs. O/C, <0.001 A vs. N, < 0.05

[a] The visual analog scale is measured on the horizontal, with a maximum value of 100 mm.

TABLE III Relationship between visual analog scale values and plasma levels of appetite peptide regulators

	CCK	*NPY*	*Ghrelin*	*Leptin*	*TNFα*	*NO_3*
Desire to eat before lunch			0.6 [b]	–0.4 [a]	–0.4 [a]	0.54 [b]
Desire to eat after lunch		0.46 [a]			–0.38 [a]	0.4 [a]
Hunger before lunch	–0.41 [a]		0.66 [b]	–0.6 [b]	–0.65 [b]	0.56 [b]
Hunger after lunch			0.55 [b]			
Fullness after lunch		–0.46 [a]	–0.4 [a]			–0.6 [b]
Prospective consumption before lunch		0.48 [a]	0.7 [b]	–0.34 [a]	–0.48 [a]	0.5 [b]
Prospective consumption after lunch					–0.43 [a]	
Palatability	–0.5 [a]		0.6 [b]	–0.4 [a]		0.38 [a]

[a] $p < 0.05$.
[b] $p < 0.01$.
CCK = cholecystokinin; NPY = neuropeptide Y; TNFα = tumor necrosis factor alpha.

of appetite by an unknown mechanism and stimulation of GH–anabolic action (15).

We have successfully used recombinant GH (rGH) to treat malnourished patients on dialysis (16). Notably, treatment with rGH was associated with a dramatic reduction in plasma levels of leptin and in insulin resistance, supporting the possibility of a direct effect on ghrelin (17). Our present results—a positive association between ghrelin and GH, and a negative association between ghrelin and leptin and insulin—support that idea.

Importantly, we found no association between ghrelin and proinflammatory cytokines, which would be the result of independent ghrelin–GH anabolic mechanisms. Moreover, GH release is altered in end-stage renal disease (18), and cytokines may be one of the mechanisms implicated in that alteration (19).

We could consider ghrelin accumulation to be positive. Moreover, RRF may play a crucial role as a

protective factor against anorexia. Because ghrelin depends on GH release for its anabolic effect, and because uremia alters that cycle, with a resulting retention of many anorexigenic molecules, the final result is anorexia.

Conclusions

Ghrelin plasma levels are elevated in PD patients. Uremic patients with anorexia show relatively lower ghrelin plasma levels than the levels seen in obese patients or in patients with normal appetite. The disordered insulin and GH metabolism in PD patients probably affects the role of ghrelin modulation in appetite control.

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Eating Behavior Disorders in Uremia: A Question of Balance in Appetite Regulation

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ABSTRACT

Eating and appetite disorders are frequent complications of the uremic syndrome which contribute to malnutrition in dialysis patients. The data suggest that uremic anorexia may occur with or without abdominal and visceral fat accumulation despite a lower food intake. This form of obesity (i.e., with low food intake and malnutrition) is more common in dialysis patients than obesity with high food intake. This article reviews the current knowledge regarding mechanisms responsible for appetite regulation in normal conditions and in uremic patients. Anorexia in dialysis patients has been historically considered as a sign of uremic toxicity due to ''inadequate'' dialysis as judged by uncertain means (''middle molecule'' accumulation, Kt/V, ''peak-concentration hypothesis,'' and others). We propose the tryptophan-serotonin hypothesis, based on a uremia-induced disorder in patients' amino acid profile—low concentrations of large neutral and branched-chain amino acids with high tryptophan levels. A high rate of tryptophan transport across the blood-brain barrier increases the synthesis of serotonin, a major appetite inhibitor. Inflammation may also play a role in the genesis of anorexia and malnutrition. For example, silent infection with *Helicobacter pylori* may be a source of cytokines with cachectic action; its eradication improves appetite and nutrition. The evaluation of appetite should take into account cultural and social aspects. Uremic patients showed a universal trend to carbohydrate preference and red meat refusal compared to healthy people. In contrast, white meat was less problematic. Uremic patients also have a remarkable attraction for citrics and strong flavors in general. Eating preferences or refusals have been related to the predominance of some appetite peptide modulators. High levels of cholecystokinin (CCK) (a powerful anorexigen) are associated with early satiety for carbohydrates and neuropeptide Y (NPY) (an orexigen) with repeated food intake. Obesity and elevated body mass index often falsely suggest a good nutritional status. In uremic patients (a hyperinsulinemia state), disorders in the regulation of fat distribution (insulin, leptin, insulin-like growth factor [IGF]-1, fatty acids, and disorders in receptors for insulin, lipoprotein lipase, mitochondrial uncoupling protein-2, and β_3-adrenoreceptors) may cause abdominal fat accumulation without an increase in appetite. Finally, appetite regulation in uremia is highly complex. Disorders in adipose tissue, gastrointestinal and neuropeptides, retained or hyperproduced inflammatory end products, and central nervous system changes may all play a role. Uremic anorexia may be explained by a hypothalamic hyperserotoninergic state derived from a high concentration of tryptophan and low branched-chain amino acids.

There is no doubt about the deleterious effects of malnutrition in dialysis patients. Malnutrition is a common factor in the two major causes of death, cardiovascular and infectious diseases, among uremic patients (1). There is a substantial body of literature on the pathophysiology of malnutrition in end-stage renal disease (ESRD) and there is general agreement on the subject's complexity. No effective standard therapeutic measures have been derived from our knowledge base. One reason for this could be the lack of alternatives to the basic need for an adequate eating behavior, a sine qua non to achieving an adequate nutritional status (2).

Eating behavior is a complex phenomenon that results from sociocultural and biologic components, complicated in uremic patients by the retention of catabolism products and profound metabolic alterations (3,4). Evaluating eating behavior disorders (EBDs) in nonuremic patients utilizes well-defined diagnostic criteria (5). However, these criteria have not been validated in dialysis patients. As a consequence, tools to assess nutrition in ESRD patients lack validated scales to define appetite disorders in terms of quality and intensity.

Our group has been interested in studying this unexplored field since 1995 in the belief that the first requirement of adequate nutrition is an appropriate appetite. We have studied the EBDs of many dialysis patients and will, in this article, review current knowledge about mechanisms responsible for appetite regulation,

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propose a classification of EBDs in dialysis patients, and suggest new therapeutic approaches for the loss of appetite.

Normal Appetite Control Regulation

Appetite is regulated by a heterogeneous feedback system that includes various signals derived from the gastrointestinal tract and liver, circulating nutrients, fat reserves and cell products (leptin, resistin), and the central nervous system (CNS). Inputs from the peripheral nervous system, including the vagus nerve and gastric receptors, are also important. Signals are integrated in the CNS, inducing hunger or satiety. The hypothalamus contains the feeding (hunger) center (lateral hypothalamus), which has dopaminergic predominance, and the satiety center (ventromedial hypothalamus), which has serotoninergic and adrenergic neurotransmitter predominance (6). High concentrations of serotonin cause satiety.

There are four phases responsible for regulating food intake:

The gastric phase starts when a meal reaches the stomach by stimulating satiety signals. Meal volume induces distention and stimulates baroreceptors; meal composition (protein and carbohydrates) stimulates cholecystokinin (CCK) release by duodenal cells which inhibits gastric motility. Central satiety (CNS) via the vagus nerve is induced by both signals (7).

In the postabsorptive phase, satiety is induced by metabolic end products, such as glucose, amino acids, and fatty acids (6–8). Their absolute and relative plasma concentrations regulate the hunger sensation. It is known that the equilibrium between tryptophan and branched-chain amino acids (BCAA) in plasma and cerebrospinal fluid plays a major role in the hunger-satiety cycle (6–8). At the same time, circulating nutrients and metabolic end products regulate appetite by inducing gastrointestinal peptide release (CCK, glucagon, and insulin) (9).

Liver vagal glucoreceptors have a physiologic role in appetite regulation. Their activation by glucose increases intracellular adenosine triphosphate (ATP) concentration, which stimulates the vagus nerve and inhibits appetite (10). Hepatocytes are sensitive to abnormal stimuli (such as cytokines) that diminish glucose and protein synthesis, exaggerating satiety and favoring cachexia (11).

Peripheral signals are integrated in the CNS (central phase), stimulating the activation of peptides and neurotransmitters. Two principal peptides, CCK and neuropeptide Y (NPY), are involved in appetite control: CCK as an anorexigen (9) and NPY as the most potent appetite stimulant known (12). In abnormal conditions, excess plasma cytokine levels (tumor necrosis factor [TNF]-α and interleukin [IL]-1) entering the CNS increase serotonin and induce satiety (13,14). Nine TNF-α molecular fragments have been isolated, each one with different actions. The 69–100 fragment has suppressive effects on food intake (15). IL-1 may also contribute to the anorexigenic effect of CCK and TNF-α by stimulating tryptophan and serotonin (16,17).

Cerebrospinal fluid amino acids are also implicated in peripheral and central appetite control. Centrally amino acids can act as neurotransmitters or precursors of neurotransmitters inducing hunger or satiety (18). The final product of these stimuli, catecholamines and serotonin, represent the ultimate mediators in the hypothalamic regulation of feeding. Catecholamine synthesis is influenced by brain levels of its precursor, tyrosine, while serotonin is locally synthesized from its precursor, tryptophan. Increasing brain serotonin activity is highly important in appetite reduction. Tryptophan concentration in cerebrospinal fluid is determined by BCAA plasma concentration, which compete with tryptophan for brain entry (18,19). Therefore two factors play a pivotal role, the concentration of free tryptophan (not bound to albumin) (FTRP) and the molar ratio between FTRP and BCAA.

Nitric oxide (NO), an endothelium-released factor synthesized from L-arginine by a specific synthase (NOS), also participates in hunger-satiety control. Interesting observations have demonstrated an interaction between brain NO levels and serotonin synthesis (20). Brain NO inactivates brain tryptophan hydroxylase (TPH), decreasing brain tryptophan and subsequently serotonin and catecholamine levels (21).

On the other hand, high brain leptin and a low NPY concentration stimulate a recently described appetite suppression pathway, the menocortin pathway. By stimulating α-melanocyte-stimulating hormone (α-MSH) and inhibiting a powerful central orexigen, the agouti-related protein (AgRP), this pathway has been demonstrated to induce an important reduction in food intake (22).

With this summary in mind, we can now review appetite regulation in uremia.

Appetite Regulation in Uremia

Even under normal conditions, appetite regulation is a very complex phenomenon; the uremic status produces a still greater degree of complexity. In uremia, multiple factors that are present cause a variety of EBDs. These factors include disorders in adipose tissue, gastrointestinal and neuropeptides, and the central nervous system (Fig. 1). Table 1 lists the neuropeptides implicated in food intake regulation. Table 2 shows the potential causes of EBDs in dialysis patients.

Anorexigen/Orexigen Imbalance

Dialysis patients have higher than normal CCK plasma levels, possibly due to renal retention or hyperproduction (23,24), while NPY plasma levels are normal or low in anorexic peritoneal dialysis (PD) patients. Recent studies have shown that CCK increases hypothalamic serotoninergic activity (via tryptophan pathway), an effect that NPY usually blocks, inducing hunger (18). In uremia, the balance of these interactions favors an anorexic effect (Fig. 2).

Accumulation of endogenous NO inhibitor, asymmetrical dimethyl-L-arginine (ADMA), has been

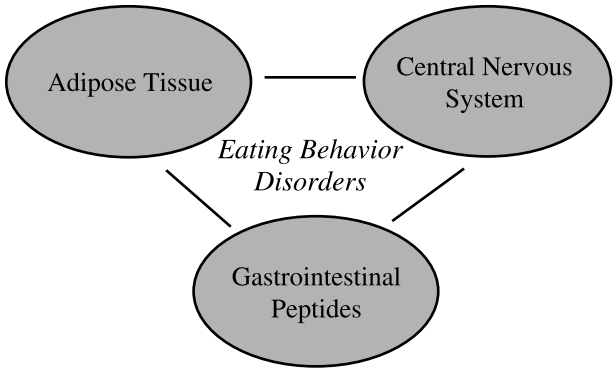

FIG. 1. EBDs result from abnormalities in gastrointestinal peptides, adipose tissue, or central nervous system appetite modulators.

described in uremic patients (25). Experimentally the administration of L-NAME (NO inhibitor) increases blood pressure and brain serotonin concentration, induces gastric distention, and increases and facilitates oxidation of L-arginine to ornithine (20).

Leptin, produced by adipocytes, regulates body fat mass through a central satiety effect. Hyperleptinemia has been described in uremia, possibly due to uremic retention, hyperproduction, or both (26). It has been suggested that leptin might be involved in malnutrition in dialysis patients. Leptin induces appetite suppression by reducing hypothalamic NPY levels by increasing sympathetic activity and hyperinsulinemia (27). Consistent with this hypothesis is the sympathetic hyperactivity (i.e., dopamine, norepinephrine, serotonin) described in uremic patients (28). Moreover, in rats, the injection of leptin decreases brain neural nitric oxide synthase (nNOS), thyrotropin-releasing hormone, and corticotropin-releasing hormone (CRF) mRNA in paraventricular and supraoptic nuclei, with the consequent increase in diencephalic serotonin content (29). In HD patients, high plasma levels of another important central anorexigen, α-MSH (30), have recently been described.

TABLE 1. Neuropeptides implicated in food intake control

Molecules	Regulated by adiposity signals	Situation in uremic patients
Orexigen		
NPY	Down-regulation	Variable (23,63,70)
AgRP	Down-regulation	Not studied
MCH	Down-regulation	Not studied
Hypocretin 1 and 2	Down-regulation	Not studied
Galacin	?	Not studied
Norepinephrine	?	Not studied
Ghrelin	?	Elevated (*)
Anorexigenic		
α-MSH	Up-regulation	Elevated (30)
CRH	Up-regulation	Normal or elevated (63)
TRH	Up-regulation	Not studied
CART	Up-regulation	Not studied
IL-1β	Up-regulation	Elevated (14,23,63)
TNF-α	Up-regulation	Elevated (14,23)
Urocortin	?	Not studied
Glucagon-like peptide 1	?	Not studied
Oxytocin	?	Not studied
Neurotensin	?	Not studied
Serotonin	?	Elevated (39,41–43)
CCK	?	Elevated (23,24)
PYY	?	Not studied
Ghrelin	?	Elevated[a]

NPY, neuropeptide Y (we found relatively low NPY values in anorectic PD patients); AgRP, agouti-related protein; MCH, melanin-concentrating hormone; α-MSH, α-melanocyte-stimulating hormone; CRH, corticotropin-releasing hormone; TRH, thyrotropin-releasing hormone; CART, cocaine and amphetamine-related transcript; CCK, cholecystokinin; PYY, polypeptide YY.

[a]Unpublished data by our group.

TABLE 2. Causes of EBDs in uremic patients

Taste abnormalities
Acuity
Metal flavor
Dry mouth
Gastric phase
Disturbances in peripheral and autonomic nervous system (axonal vagus nerve degeneration)
GIT motility disorders associated with peptide, TNF-α or uremia per se, and diabetic gastropathy
Abdominal discomfort in PD patients
Gastric distention associated to NO deficiency (accumulation of ADMA)
Postabsorptive phase
PD solution based on glucose or amino acids (peritoneal absorption)
Inappropriate diets (rich in carbohydrates)
Hepatic phase
Disorders in metabolic hepatic glucose (increase in glycogenolysis and decrease in gluconeogenesis)
High cytokine plasma levels decreasing hepatic ATP production
Central (brain) phase
High plasma levels of satietins: peptide (CCK, glucagon, insulin, leptin, cortisol release factor, pancreatic polypeptide, leptin, α-MSH), cytokines (TNF-α and IL-1), lower zinc, and possibly NPY[a]
Lower NO synthesis (accumulation of ADMA)
High FTRP producing an increase in serotonin synthesis by NO deficiency
Imbalance in brain amino acids (high FTRP:BCAA ratio)
Fat store metabolism
Insulin resistance (leptin, resistin, fatty acids, TNF-α) which induces abnormal regulation of intracerebral NPY
Other causes: low doses if dialysis, infections, medications, depression, poverty, alcohol abuse, rigorous diet before starting dialysis

GIT, gastrointestinal tract; TNF-α, tumor necrosis factors α; PD, peritoneal dialysis; NO, nitric oxide; ATP, adenosine triphosphate; CCK, cholecystokinin; α-MSH, α-melanocyte-stimulating hormone; IL-1, interleukin 1; NPY, neuropeptide Y; FTRP, free tryptophan; BCAA, branched-chain amino acids.

[a]Another group found high plasma levels (70). In our study (23), NPY was normal in 80% of the cases; relatively low values were found in anorectic PD patients.

Particular Characteristics in Dialysis Patients

In PD, the use of glucose or amino acid dialysate makes patients particularly sensitive to the influence of appetite regulation mechanisms. The peritoneum provides a rapid way for these elements to reach the bloodstream (7,8). Furthermore, abdominal distention induced by peritoneal fluid and high osmolality may cause food intake disregulation (31). However, an increase in intragastric pressure has not been demonstrated in these patients (32).

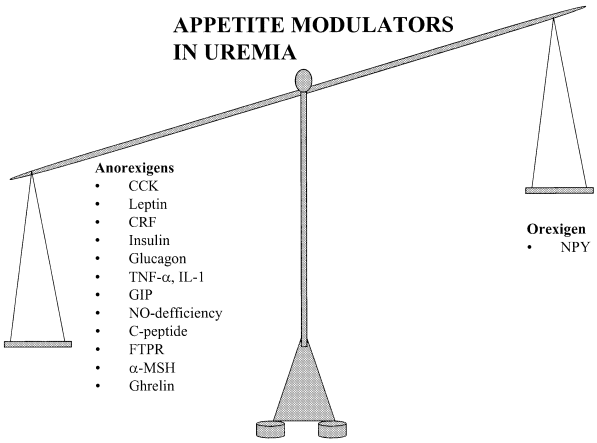

FIG. 2. Imbalance between anorexigen versus orexigen substances in uremia status. CCK, cholecystokinin; CRF, corticotropin-releasing hormone; TNF-α, tumor necrosis factor α; IL-1, interleukin 1; GIP, gastric inhibitory peptide; NO, nitric oxide; FTRP, free tryptophan; NPY, neuropeptide Y; gherlin: elevated (Aguilera et al., unpublished data).

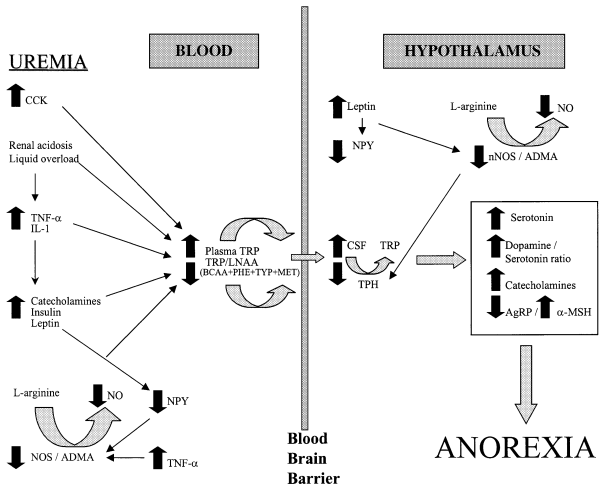

FIG. 3. Tryptophan/serotonin disorder hypothesis. CCK, cholecystokinin; IL-1, interleukin 1; TNF-α, tumor necrosis factor α; TRP, tryptophan; LNAA, large neutral amino acids; BCAA, branched-chain amino acids; NO, nitric oxide; NPY, neuropeptide Y; NOS, nitric oxide synthase; nNOS, neural nitric synthase; ADMA, asymmetric dimethyl-L-arginine; CSF, cerebrospinal fluid; TRH, tryptophan hydroxylase; α-MSH, melanocyte-stimulating hormone; AgRP (hypocretin), agouti-related protein.

Peripheral and autonomic nervous system dysfunction with axonal vagus nerve degeneration is a scantily studied complication of uremia (33). It has the potential to inhibit gastrointestinal motility with an adverse effect on appetite.

Dialysis Dose

Many studies have shown a strong correlation between Kt/V_{urea} and daily protein intake, though residual renal function may be more important for appetite than dialysis dose (34). These correlations suggest that one or more molecules that inhibit appetite accumulate in dialysis patients. However, the clinical correlation between dialysis dose and the presence of EBDs is poor (35–38). Therefore it is impossible to account for the EBDs of uremia exclusively through low doses of dialysis.

Amino Acid Profile Disorders in Uremia

Brain serotonin overproduction may be the final common pathway involved in the genesis of EBDs in dialysis patients (36). Serotonin is the final element in hunger genesis; low levels induce hunger, while with increased levels satiety appears (18,19,21,39,40). In uremic patients, high plasma and brain levels of tryptophan, the amino acid precursor of serotonin, have been repeatedly reported. Increases in plasma aromatic amino acid concentration (including tryptophan) and reductions in neutral amino acids (consequently increasing the FTRP/BCAA ratio) have also been reported in this population (40–43) as well as in cirrhotic patients (44). The high plasma and intracerebral concentration of FTRP and, as a consequence, serotonin, increase the serotonin/dopamine ratio. In uremia, this phenomenon is perpetuated by numerous factors including high concentrations of IL-1, TNF-α, CCK, leptin, catecholamines, insulin, interdialysis fluid overload releasing cytokines, metabolic acidosis, and low levels of NO induced by high ADMA concentrations (Fig. 3).

These disturbances probably induce a poor response to peripheral appetite-stimulating signals. The clinically variable expression of uremic anorexia could be the result of different combinations of changes in amino acid profiles and peripheral appetite modulators (CCK, NPY, leptin, cytokines, and NO). Consistent with our tryptophan/serotonin disorders hypothesis, Hiroshige et al. (45) gave BCAA to malnourished HD patients and observed an improvement in appetite and nutritional status.

Inflammation

Tumor necrosis factor α and IL-1, elevated in the plasma of dialysis patients with anorexia and malnutrition (23), interfere with the mechanism regulating the plasma tryptophan pool. Inflammatory molecules oxidize BCAA, increasing FTRP, which in turn facilitates tryptophan entry into the brain and serotonin production (13,18,46). TNF-α also decreases gastrointestinal motility, another potential mechanism inducing anorexia (47). At the same time, TNF-α decreases NOS levels (through selective destabilization of mRNA transcription) and increases sympathetic activity inducing anorexia (48). Thus uremic NO deficiency could be another cause of anorexia via inactivation of brain TPH (21).

Another interesting hypothesis proposed by Stevinkel et al. (49), the malnutrition, inflammation, and atherosclerosis (MIA) hypothesis, suggests that anorexia results from inflammation. They propose two specific forms of malnutrition: MIA type I with normal or low serum albumin, usually absent comorbidity and inflammatory markers, low food intake, normal resting energy expenditure (REE), increased oxidative stress, and decreased

protein catabolism; it is potentially reversible with dialysis and nutritional support. Type II is associated with low albumin, high comorbidity and inflammatory markers, low or normal food intake, elevated REE, markedly elevated oxidative stress, and increased protein catabolism; it is not reversible with dialysis or nutritional support.

Our observation that elevated cytokine levels are associated with anorexia and malnutrition in PD patients is consistent with the MIA hypothesis (23). Moreover, TNF-α can inhibit hepatic albumin gene expression (contributing to dialysis protein malnutrition) and induce systemic acidosis and muscle mass wasting with structural protein breakdown (22,50,51). Also supporting the MIA hypothesis are our findings in a group of PD patients suffering from anorexia and *Helicobacter pylori* infection (52). These patients showed high plasma cytokine levels and low anorexigen substances and residual renal function (RRF). After *H. pylori* eradication, appetite improved, orexigen and biochemical nutritional markers increased, and cytokine levels decreased. Another group of patients having *H. pylori* infection without anorexia appeared to be protected from the cachectic actions of cytokines by their RRF.

Evaluation of EBDs in Dialysis Patients

The evaluation of appetite should take into account cultural and social considerations, such as food preferences, gender, and habits. To quantify appetite, questionnaires utilizing scales on eating motivation and diet can be utilized. The appetite of dialysis patients has been of little interest to those studying appetite in general, as well as those studying nutrition in ESRD patients. For these reasons we felt prompted to explore the area of appetite in dialysis patients.

Eating Preference and Refusal in Dialysis Patients

By diet survey, we studied eating preferences and food intake in 48 PD patients and 36 healthy subjects from our region, matched for age and gender (Aguilera A, et al., unpublished data). Dialysis patients showed a universal trend toward carbohydrate preference and red meat refusal (veal, lamb, pork) relative to healthy people. White meat (poultry, turkey, fish) was better accepted among patients. They confessed to a marked attraction to citrics and strong flavors in general. Similar findings have been reported by Dobell et al. (53). Other authors (54) have demonstrated taste abnormalities (including bitter impairment in PD patients and a salty taste in HD patients) associated with EBDs.

Taste is an important factor that regulates food preference and the amount of food eaten (55). In several EBDs, taste disorders and meal flavors have been identified as risk factors for decreasing food intake and changing food preferences (56,57). The influence of taste abnormalities on EBDs in uremic patients has not been studied. We have seen that in patients with a clearly insufficient dialysis dose (typically with uremic halitosis), taste abnormalities (acuity, metal flavor, and dry mouth) undoubtedly play a role in the quantity of food consumed.

Some eating preferences have been related to serum levels of peptide appetite modulators. In particular, high CCK levels are associated with early satiety for carbohydrates (58). Little information is available on modulators of protein intake. Furthermore, none of these links have been considered in dialysis patients.

Eating Motivation

Eating motivation can be evaluated through an eating motivation scale (visual analog scale [VAS]) (59) that has been validated for dialysis patients (31,60,61). The VAS includes five questions which are answered at least 4 hours before (in fasting conditions) and immediately after eating. It measures desire, hunger and fullness, prospective consumption, and palatability. The results are measured on a scale from 0 to 100, added, and divided by five, giving a score. Values greater than 60 (before eating) are considered normal in a healthy population (52,59). The VAS has successfully been used in uremic patients (31,60,61). Wright et al. (62) recently published another model to evaluate appetite disorders in HD patients.

Table 3 shows the results of the VAS in different groups of dialysis patients suffering from EBDs. Motivation to eat was significantly lower in patients with uremic anorexia than in asymptomatic or obese patients. Obese patients show the highest eating motivation value, the highest food intake, and the highest early and later peaks of NPY (explaining hunger sensation). Our data on the VAS have been validated by the significant correlation between greater values of the scale and higher NPY (range 0.51 to 0.55) and lower CCK (range -0.43 to -0.66) plasma levels. Notably the metabolic disorder caused by CCK- and NPY-releasing abnormalities in uremic status is not expected to be correct by the current renal replacement therapies.

Diet Survey

We have determined mean daily dietary intake from individual 24-hour food records over a 3-day period in 68 patients on PD and 54 on HD. Daily calorie, carbohydrate, lipid, and protein intakes were calculated for each patient using a commercially available computer software (Wander; Sandoz Nutrición, Barcelona, Spain). Types of macronutrients ingested and the most frequent (preferred) macronutrients were estimated by expert personnel. In general, PD patients showed a lower daily food intake (kcal/day) than those on HD (1867.6 ± 332.3 versus 2173 ± 297, $p < 0.05$). However, when we included glucose absorbed by peritoneum, PD patients showed a higher caloric intake than those on HD (2380.6 ± 274 kcal/day, $p < 0.05$). These differences were mainly based on carbohydrate intake: PD patients (245.5 ± 43.9) versus HD patients (186 ± 38 kcal/day) ($p < 0.05$). The protein intake was also significantly lower in PD versus HD patients (77.5 ± 16.1 versus 89.3 ± 12, $p < 0.05$). Our results highlight the negative effect of peritoneal glucose absorption, inducing

TABLE 3. Eating motivation measured through the VAS in PD patients suffering from EBDs

VAS	Anorectic (n = 12)	Obese (n = 12)	Asymptomatic (n = 18)	Controls (n = 10)	p
Diet survey (kcal/day)	1277 ± 467.4 (a,b,c)	2320 ± 179.4 (a)	2006 ± 351 (b)	2089 ± 339 (c)	(a) < 0.01, (b,c) < 0.05
Proteins (kcal/day)	63 ± 18 (d)	85.7 ± 16.6 (d)	83.8 ± 13.7	74.5 ± 21.8	(d) < 0.05
Desire to eat before lunch	60 ± 6.1 (e,f)	76.6 ± 6 (e)	67.8 ± 6.9	72.8 ± 3.9 (f)	(e,f) < 0.01
Desire to eat after lunch	8.6 ± 2.2 (g)	21.6 ± 4 (g)	13.2 ± 5	13.5 ± 8.5	(g) < 0.05
Hunger before lunch	60 ± 6.1 (h,i,j)	78.3 ± 6 (h)	68.6 ± 4.7 (i)	74.3 ± 4.5 (c,j)	(h) < 0.001, (i,j) < 0.01
Hunger after lunch	8 ± 4.4 (k,l)	21.6 ± 4 (k)	12.8 ± 5.5	17.1 ± 4.8 (l)	(k,l) < 0.01
Fullness before lunch	28 ± 8.4 (m,n)	18.8 ± 2.5	12.5 ± 4.2 (m)	11.8 ± 4.1 (n)	(m,n) < 0.01
Fullness after lunch	81 ± 5.4 (o)	59.1 ± 19.6 (o,p)	77 ± 5.6	77 ± 5.6 (p)	(o,p) < 0.05
Prospective consumption before lunch	59 ± 5.5 (q,r)	78.3 ± 4 (s)	71.4 ± 3.7 (q,s)	75.7 ± 4.5 (r)	(q,r) < 0.001, (s) < 0.01
Prospective consumption after lunch	6 ± 2.2 (t,u,v)	25 ± 5.4 (t,w)	12.3 ± 2.7 (u,w)	13.5 ± 4.7 (v)	(t) < 0.001, (u,v) < 0.01
Palatability	60 ± 7 (x,y,z)	75 ± 5.4 (x)	71.4 ± 4.7 (y)	74.3 ± 5.3 (z)	(x,y,z) < 0.01

VAS is measured on a horizontal scale with a maximum value of 100.
(a–x): statistical differences (read in horizontal).

satiety and inhibiting protein intake. Table 3 shows the daily food intake in patients with EBDs on PD. Anorexic patients showed a severe lack of eating motivation and the lowest food and protein intake.

We have had good results using these instruments in different studies (52; Aguilera A, et al., unpublished data).

Biochemical Appetite Modulation in Uremia

The list of factors influencing appetite in uremia (Fig. 2) show a clear trend to ''incline the balance'' toward anorexia. The high plasma levels of several anorexigen substances including CCK, insulin, glucagon, leptin, anti-NO activity, cytokines, tryptophan-serotonin, and others (14,23,27,30,39,43,48) probably cannot be compensated for by high plasma levels of ghrelin (Aguilera A, et al., unpublished data) and the normal or low NPY plasma levels (23). However, there is quite a substantial disparity among patients in their loss of appetite.

This observation led us to perform a study (62; Aguilera A, et al., unpublished data) to examine appetite modulators in dialysis patients. We divided our patients into three groups: anorectics, obese with a tendency to bulimia, and patients without EBDs. We determined serum appetite modulator substances at baseline and 30, 60, and 90 minutes after a standard food stimulus (Fresubin; Fresenius, Spain; with 750 cc, 750 kcal, 17 g of carbohydrate, 7.5 g of protein, 5.8 g of fat, and 79 ml of water). Eating motivation was evaluated by the VAS (59). Healthy controls showed an early peak of NPY, with hunger persisting 60 minutes after food stimuli. In contrast, patients with anorexia showed a higher satiety before and after eating, and a low desire for and pleasure from eating. At baseline, anorectic patients showed high levels of C-peptide, CCK, IL-1, TNF-α, and gastric inhibitory peptide (GIP). These anorexigens showed an early peak that remained elevated during the observation period. NPY levels were low without an early peak after eating. In fact, the NPY stimulation curve was flat, with levels decreasing to values lower than baseline at 90 minutes in association with less appetite. The early plasma peak of CCK may explain the early satiety of these patients, also demonstrated by the VAS (63). The obese group showed higher baseline NPY levels, lower levels of anorexigens, and a prolonged hunger sensation measured by VAS compared with uremics not suffering from EBDs and with anorectic patients (63).

Hyperleptinemia and low nitrate (NO_3) values are also anorexigen agents. According to our results, leptin is an anorexigen only at baseline, not after food intake. NO_3 was lower in all studied patients relative to controls (63; Aguilera A, et al., unpublished data).

The change in NPY and insulin levels after food intake were parallel in all patients, but in obese patients they were accompanied by a premature and prolonged hunger sensation. The high insulin levels reflect the carbohydrate intolerance in uremia (and in some cases, obesity). We postulate that the abnormal secretion of NPY in obese patients may be conditioned by peripheral insulin resistance (63).

Proposal for Classification of EBDs in Uremic Patients

Uremic Anorexia

Diagnostic Criteria for Anorexia. In previous studies we have defined anorexia as low eating motivation (personal interview and VAS < 60) (52,63), low food intake (daily dietary assessment < 30 kcal/kg/day), and low nutritional markers (64).

Classification Depending on the Period of Appearance. Early anorexia appears in the first months of dialysis. It is generally associated with a low dialysis dose or, on some occasions, with systemic inflammation. The treatment required is an increase in the dialysis dose, nutritional support, and identification and treatment of any inflammatory disorders. Late anorexia generally appears in patients who have received long-term dialysis. These patients are often anuric and may have other complications of uremia. Any inflamed organ (including anuric kidneys) may be a source of cytokines with anorexigen and cachectic actions; thus

it is necessary to rule out silent and chronic infections. The treatment should include anorexigen agents such as megestrol acetate, anabolic agents such as nandrolone decanoate (mainly in men more than 55 years old) (65) and recombinant human growth hormone (hGHr), potential anticytokine agents (antibodies, anti-TNF-α, thalidomide), and nutritional support. Steroidal and nonsteroidal anti-inflammatory drugs are anecdotally suggested.

Classification Based on Levels of Catabolism. The first classification is associated with hypercatabolism, severe malnutrition, high levels of inflammatory markers, urea and oxidative stress, increased REE, and metabolic acidosis. It is nonreversible with dialysis or nutritional support and represents the type II MIA syndrome. Recently the administration of pentoxifylline, an anti-TNF-α agent, decreased whole-body proteolysis in uremic patients (66).

The second classification has no associated hypercatabolism, with normal or low serum albumin, uncommon comorbidity, absence of inflammatory markers, low food intake, normal REE, increased oxidative stress, and decreased protein catabolism. It is potentially reversible with dialysis and nutritional support, and represents the type I MIA syndrome.

Classification Depending on Persistence. Cyclic anorexia appears sporadically and requires that acute dental, respiratory, urinary (especially in anuric patients), old vascular graft, and gastrointestinal infections be sought. Treatment consists of eradicating any focus of infection.

Persistent anorexia, usually in long-term dialysis patients, may be associated with chronic infections (dental, *H. pylori* carrier, old vascular access, urinary) and chronic rejected kidney. It is also important to consider neoplasia as an etiology. The treatment should or may include orexigens (megestrol acetate), anabolic agents (nandrolone decanoate), anticytokine agents (66), and anti-inflammatory drugs, including steroids; nutritional support as well is required.

Early Satiety

While generally associated with anorexia, it is occasionally an isolated symptom present in patients who do not meet the full criteria for the diagnosis of anorexia. Patients show normal eating motivation, but they suffer satiety after eating a small quantity of food. In our study of these patients we detected an early (30 minutes after food stimuli) CCK plasma peak, very different from healthy controls in whom this peak does not appear. This finding suggests the potential value of using CCK inhibitors (proglumide, loxiglumide) prior to mealtime (67).

Obesity with Malnutrition

Obesity with malnutrition is generally associated with protein malnutrition (Kwashiorkor-like syndrome) and is a consequence of high carbohydrate and low protein intake. It occurs more frequently in PD patients and type II diabetics, with insulin resistance, dyslipidemia, and high cardiovascular risk.

Clinically it is characterized by a high body mass index (BMI). According to the World Health Organization (WHO) criteria (68), obesity grade I characterized by a BMI of 25–30 kg/m^2, grade II 30–40 kg/m^2, and grade III greater than 40 kg/m^2, low or normal eating motivation (VAS > 60, low or normal quantities of daily food intake (by diet survey), and frequently DSM-IV criteria (69).

In this group of patients, obesity and a high BMI falsely suggest a good nutritional status. In normal conditions, abdominal visceral fat accumulation and female fat distribution are regulated by plasma levels of sexual hormones, insulin, insulin-like growth factor (IGF)-1, leptin, fatty acids, as well as disorders in insulin receptors and the β_3-adrenoreceptor system (70,71). With hyperinsulinemia, abnormalities in insulin receptors, high fatty acids, TNF-α, and leptin plasma levels produce a vicious circle with more carbohydrate intolerance and more insulin production, resulting in a decrease in the abdominal insulin-lipolytic capacity, and as a consequence, abdominal obesity (72).

In uremic patients, the mechanisms responsible for regulating the fat distribution are dysfunctional (26,28,73–76). For example, there is a uremic-acquired gene disorder in leptin and a mitochondrial uncoupling protein called UCP2 that is responsible for fat accumulation in PD patients (77). In HD patients the fat accumulation in the first year of HD is associated with malnutrition (78). We speculate that abdominal fat accumulation in dialysis patients without increased appetite might be explained through both uremic hyperinsulinism, hyperparathyroidism (decreasing the fat-lipoprotein lipase [LPL] activity) (76,79,80) and acquired fat gene disorders (leptin, β_3-adrenoreceptors, UCP2, LPL, and transforming growth factor [TGF]-β) (73,75,77,81,82) (Fig. 4).

On the other hand, we have found (63; Aguilera A, et al., unpublished data) that on some occasions obesity

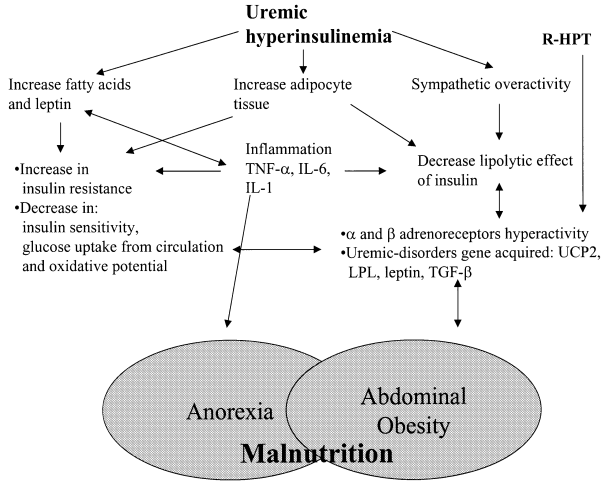

FIG. 4. Hypothesis about the influence of uremic hyperinsulinism and the pathogenesis of central obesity with malnutrition. R-HPT, renal hyperparathyroidism; UCP2, mitochondrial uncoupling protein; LPL, lipoprotein lipase.

in dialysis patients may be associated with high food intake (equivalent to bulimia).We have found plasma levels of NPY to be high at baseline, with two peaks after food stimuli. Since insulin is responsible for regulating NPY release, insulin resistance and large fat stores in obese patients play an important role in appetite regulation in these patients (Table 1), as has been suggested in other circumstances (22).

In conclusion, we would like to propose that adipose tissue is the protagonist in appetite changes induced by uremia. Uremic carbohydrate intolerance (represented by elevated plasma insulin levels and mediated by increased levels of TNF-α, free fatty acids, leptin, and other molecules of adipose origin) induces different EBDs. The regulation of the normal set point for appetite, a result of the equilibrium between central and peripheral stimuli (peptides, neurotransmitters), is broken. The variability in these factors in dialysis patients account for their different EBDs. Uremic anorexia may be explained by a hypothalamic hyperserotoninergic state resulting from a high concentration of tryptophan, peripheral stimuli, and a low levels of branched-chain amino acids.

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Original Article

Anorexigen (TNF-α, cholecystokinin) and orexigen (neuropeptide Y) plasma levels in peritoneal dialysis (PD) patients: their relationship with nutritional parameters

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Abstract

Background. Malnutrition has definitely been related to mortality among dialysis patients. Persistent loss of appetite is one of the major symptoms found in these patients. It is also well recognized that several substances produce anorexia or disorders of the hunger–satiety cycle in several diseases. The aim of this study was to identify the role of anorexigen substances (TNF-α and cholecystokinin or CCK) and an orexigen substance (neuropeptide Y or NPY) in anorexia and malnutrition among 55 clinically stable peritoneal dialysis (PD) patients.

Results. High TNF-α plasma levels were found in 41 of 42 patients (97.6%) with a mean of 70.5 ± 32.3 pg/ml. Patients with anorexia ($n=11$) or anorexia with nausea or vomiting ($n=5$) had higher TNF-α values than patients without these symptoms (75.9 ± 34 vs 52.1 ± 24.5 pg/ml, $P<0.05$). Eight patients with a prior diagnosis of acid pylori disease showed higher TNF-α values (87.2 ± 24.3) than 30 unaffected patients (63.6 ± 30.5, $P<0.05$). TNF-α showed a significant negative linear correlation with retinol binding protein (RBP) ($r=-0.37$, $n=34$, $P<0.05$), and venous pH ($r=-0.4$, $n=42$, $P<0.01$); also, TNF-α values higher than 65 pg/ml were inversely associated with transferrin, cholesterol, blood urea nitrogen (BUN) and CCK. Patients with prealbumin levels lower than 30 mg/dl, a BMI lower than 30 kg/m^2, nPCR lower than 1.1 g/kg/day and urea KT/V lower than 2.2 showed higher serum TNF-α levels. Patients who had been on CAPD treatment for longer periods showed higher TNF-α values.

High plasma CCK levels were found in 38 of 45 patients (84%), mean 45.9 ± 32.3 pg/ml. Patients with anorexia had no difference in CCK values compared with those without. A direct association was found between CCK levels and some nutritional markers (albumin, fibronectin, triglycerides, folic acid and nPCR in non diabetic patients). Although CCK has a recognized anorectic effect, this direct association might be because of an abnormal stimulation of CCK–glucose feedback (trypsin) due to continuous peritoneal glucose absorption. This suggests that CCK could be an immediate food intake marker in PD patients.

The NPY plasma levels were normal in 33 patients, high in 6 and low in 11. Patients with anorexia showed lower NPY levels than those without. NPY values greater than 50 pg/ml were directly associated with higher transferrin, prealbumin, RBP, nPCR and urea KT/V values. Importantly, a negative linear correlation between NPY and TNF-α was found ($r=-0.42$, $n=41$, $P<0.01$).

There was no significant relationship between residual renal clearance and the serum levels of the three peptides.

Conclusion. In conclusion, our data suggest that high TNF-α and low NPY serum levels are associated with anorexia. High TNF-α, low CCK and low NPY serum levels are also related to a poor nutritional status. Further research on these circulating substances is required.

Key words: nutrition; peritoneal dialysis; uremic anorexia; TNF-α; cholecystokinin; neuropeptide Y; acidosis

Introduction

Protein–energy malnutrition has been demonstrated in 20–40% of patients on maintenance dialysis [1,2]. Malnutrition has definitely been related to high morbidity and mortality both in hemodialysis (HD) [3] and peritoneal dialysis (PD) patients [4,5].

Several factors have been associated with a poor nutritional status: decreased nutrient intake (anorexia, prescribed diets, sociocultural and economic factors, depression and sleep disturbances), decreased nutrient utilization, nutrient loss, altered hormonal and enzym-

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atic function and increased metabolic demand [6]. Anorexia is possibly one of the most important causes of malnutrition in the uremic state [7]. It is considered a sign of uremic intoxication, associated with dysgeusia, nausea and vomiting; these are used as clinical indicators for initiating dialysis and also for inadequate dialysis [8]. However, it is frequent to find patients with adequate dialysis criteria who nevertheless show malnutrition associated with persistent and severe anorexia. Although the exact cause of anorexia is unknown, several factors have been suggested including: uremic toxicity, insufficient dialysis, deficient diets, gastropathy in diabetics, infection, drugs, psychosocial and economical factors and, in particular in PD patients, with abdominal discomfort and glucose absorption through the peritoneal membrane [8].

Several endogenous substances have been related to satiety–hunger cycle disorders. TNF-α and cholecystokinin (CCK) have been recognized as having an anorexigen effect under different conditions. TNF-α is associated with anorexia in wasting syndromes [9], anorexia nervosa [10], rheumatoid cachexia [11] and cancer [12]. In dialysis patients, studies on the effects of TNF-α almost never include this recognized consequence [13,14].

CCK is an intestinal peptide released by duodenal cells in the presence of carbohydrates in intestinal lumen. It has a satiety effect through central and peripheral action [15,16]. CCK has been implicated in anorexia nervosa [15,16], cancer [17], senile [18] and alcoholic anorexia [19]. CCK has renal clearance, therefore ESRD patients have high plasma levels [20–23].

Neuropeptide Y (NPY) is the most potent orexigen known in relation to the pathogenesis of obesity [24], even diabetic obesity [25]. To our knowledge, NPY plasma levels have not been related to uraemic hunger–satiety processes.

The ultimate aim of this cross-sectional study was to identify the role played by orexigen–anorexigen substances in uremic anorexia, hunger–satiety regulation and poor nutritional status.

Patients

We studied 55 clinically stable peritoneal dialysis (PD) patients, 47 on continuous ambulatory peritoneal dialysis (CAPD) and 8 on automatic peritoneal dialysis (APD) (2 continuous cycler-assisted peritoneal dialysis (CCPD) and 6 nocturnal peritoneal dialysis (NPD)), 21 male and 34 female, ranging in age from 22 to 86 years (mean 51 ± 14.2). No acute disorders were present during the 2 months prior to the study. Patients who suffered intestinal, pancreatic and chronic liver diseases, active infections, neoplasms and chronic obstructive lung disease were not included. The causes of chronic renal failure were glomerulonephritis in 12 cases (21.8%), diabetes in 11 (20%), chronic pyelonephritis in 7 (12.7%), polycystic kidney disease in 7 (12.7%), nephrosclerosis in 7 (12.7%), unknown etiology in 4 (7.2%), systemic diseases in 4 (7.3%) and congenital diseases in 3 (5.4%). The mean period on PD was 30.3 ± 34.8, (1–179) months.

Anorexia, assessed by an interview with the patient guided through a 3-day food intake survey, was present in 16 patients, isolated in 11 and associated with nausea/vomiting in 5. Twenty patients were asymptomatic and 19 referred to mild occasional gastrointestinal (GI) symptoms (dyspepsia, sporadic abdominal pain, nausea or vomiting and pyrosis). Forty-three patients (78.2%) had never been diagnosed with GI disease. Of the remainder, eight had been previously diagnosed with acid pylori disease, two with hiatus hernia and two with spastic colon and diverticulosis.

Peritoneal ultrafiltration capacity was considered normal in 43 patients (800 ± 200 ml/day, using a combination of 1.36 and 2.27% dextrose), high in 7 (using most of their bags with dextrose 1.36%) and low in 5 patients (requiring at least 25% of the daily bags with dextrose 3.86%).

Methods

For reasons outside our control, not all determinations were performed in all patients. Throughout the paper, we expressly indicate the exact figures for each analysis.

The following parameters were determined:

(1) Dialysis adequacy: urea KT/V and nPCR (normalized protein catabolic rate) [5].

(2) Long-term nutritional markers: plasma creatinine, albumin, cholesterol (colorimetric method, Hitachi 704) and transferrin by the immunonephelometric method (Boehring Nephelometer-Terminal S.A., Spain). Plasma levels of vitamin B_{12} and folic acid (radioimmunoassay), ferritin and iron (Hitachi 911) and triglycerides (Hitachi 704). Short- to medium-term nutritional markers (short half-life proteins) include plasma prealbumin, retinol-binding protein (RBP), fibronectin, antithrombin III (AT III), ceruloplasmin and α-1-antitrypsin analyzed by immunonephelometric methods. NPCR, urea nitrogen, phosphorus and potassium were also considered representative of short-term food intake markers. Body mass index (weight in kg/height^2 in m) was the nutritional anthropometric method used to evaluate severe obesity ($BMI \geqslant 30$).

(3) Hunger–satiety cycle parameters (anorexigen–orexigen substances).

TNF-α plasma levels

Five milliliters of plasma were drawn. Plasma TNF-α was measured using the enzyme-amplified sensitive immunoassay (ELISA) performed on micro-titer plates. It is based on the oligoclonal system, in which several monoclonal antibodies are used. The minimum detectable concentration is estimated to be 3 pg/ml and is defined as the TNF-α concentration corresponding to the mean of 20 replicates of the zero standard ± 2 standard deviations. It is specific as TNF-α ELISA does not cross-react with TNF-β, IL-1, IL-2 and IFN-α, β or γ. Normal values ranged from 3 to 20 pg/ml (Easia Medgenix Diagnostics S.A. Belgium).

Fifteen of the 16 anorectic patients, 13 of the 20 asymptomatic patients and 14 of the 19 with mild GI symptoms had TNF-α levels determined (42 patients). TNF-α determination was performed on 30 of the 43 patients without previous GI disease and on the 12 patients with this antecedent, 8 were diagnosed with acid pylori disease.

Cholecystokinin (CCK)

The 26–33 unsulfated fragment was determined (Peninsula Laboratories, Inc.) with an IC_{50} of 35 pg/100 µl. Specificity to CCK 26–33, CCK 33 with a percentage cross-reactivity 100%. Values considered normal were 12–20 pg/ml. CCK determination was performed in 15 of the 16 anorectic patients, 15 of the 20 asymptomatic patients and 15 of the 19 patients with other GI symptoms (45 patients). CCK was also studied in 33 of the 43 patients without prior GI diagnoses and in 12 with antecedents.

Neuropeptide Y (NPY)

The method used was radioimmunoassay (Peninsula Laboratories, Inc.). The IC_{50} was 23 pg/100 µl (normal values were 20–80 pg/ml). NPY was determined in 50 of the 55 patients studied: 14 of the 16 anorectic patients, 19 of the 20 asymptomatic and 17 of the 19 patients with other GI symptoms. NPY was also studied in 38 of 43 patients without prior GI diagnoses and in 12 with antecedents.

The statistical study was performed by Student's *t* and Mann–Whitney tests and linear regression analysis. When linear regression analysis gave no statistically significant results, a stratified analysis for different values of the three target variables was performed. The appropriate comparative test was then applied. The values are expressed as mean $\pm$ 1 SD.

Results

Table 1 shows the general analytical data, short-, medium-and long-term nutritional markers. Mean levels of cholesterol, vitamin B_{12}, ferritin, RBP and fibronectin are all over the normal range. There was no association between anorexia and peritoneal ultrafiltration capacity or peritoneal glucose load.

Table 1. General analytical parameters and short-, medium- and long-term nutritional markers in PD patients

Parameter	Mean $\pm$ SD	*n*	Normal range
Hemoglobin (g/dl)	11 $\pm$ 1.4	55	10–15
Urea (mg/l)	146.1 $\pm$ 38	55	variable
Potassium (mEq/l)	4.4 $\pm$ 0.7	55	3.5–4.5
Calcium (mg/dl)	9.5 $\pm$ 0.9	55	8.5–10.5
Phosphorus (mg/dl)	5.5 $\pm$ 1.5	55	3.5–6.5
Urea KT/V	2.2 $\pm$ 0.5	55	$\geqslant$1.8
nPCR (g/kg/day)	1 $\pm$ 0.2	55	$\geqslant$1
RBP (mg/dl)	11.1 $\pm$ 3.8*	38	3–6
Fibronectin (mg/dl)	40 $\pm$ 15.1*	41	25–40
Prealbumin (mg/dl)	32.7 $\pm$ 9.8	44	10–40
Antithrombin III (%)	100 $\pm$ 15.1	38	90–120
Ceruloplasmin (mg/dl)	26.5 $\pm$ 5.5	44	15–60
α-1-antitrypsin (mg/dl)	248.7 $\pm$ 67.6	45	150–350
Venous pH	7.34 $\pm$ 0.04	50	7.3–7.4
Cr. Clearance (ml/min)	2.14 $\pm$ 2.26	55	variable
Creatinine (mg/dl)†	9.4 $\pm$ 2.2	55	variable
Albumin (g/dl)†	3.8 $\pm$ 0.5	55	3.5–5
Triglycerides (mg/dl)	160.6 $\pm$ 90	55	40–170
Cholesterol (mg/dl)†	226.2 $\pm$ 46.8*	55	90–200
Vitamin B_{12} (pg/ml)	785.2 $\pm$ 339.4*	52	150–750
Folic acid (ng/ml)	7.5 $\pm$ 3.7	52	2–10
Iron (µg/dl)	63.7 $\pm$ 24.2	55	59–145
Ferritin (ng/ml)	253.1 $\pm$ 213.4*	55	50–250
Transferrin (mg/dl)†	255.2 $\pm$ 48.1	55	209–389

*Different from normal range.
†Long-term nutritional markers.

TNF-α plasma levels

High TNF-α levels were detected in 41 of 42 patients (97.6%), with a mean of 70.5 $\pm$ 32.3 (18.1–156.3 pg/ml) (Figure 1). PD duration was longer in patients with TNF-α levels greater than 65 pg/ml (45.8 $\pm$ 42.4 months in 22 patients vs 23.3 $\pm$ 27.2 months in the 20 patients with levels less than 65 pg/ml, $P<0.05$).

Patients with anorexia or anorexia with nausea or vomiting showed higher plasma TNF-α levels than patients without GI symptoms (75.9 $\pm$ 34, $n=15$ vs 52.1 $\pm$ 24.5 pg/ml, $n=13$; $P<0.05$). Non-anorectic patients suffering other GI symptoms also had higher levels of TNF-α than asymptomatic patients (81.7 $\pm$ 31.5, $n=14$ vs 52.1 $\pm$ 24.5 pg/ml; $n=13$, $P<0.05$).

Higher plasma TNF-α levels were found in eight patients previously diagnosed with acid pylori disease, (87.2 $\pm$ 24.3, $n=8$) compared with patients without GI disease (63.6 $\pm$ 30.5 pg/ml, $n=30$, $P<0.05$).

Table 2 shows the statistically significant differences for several nutritional parameters according to TNF-α levels greater and less than 65 pg/ml.

A significant negative linear correlation appeared between TNF-α and plasma RBP levels ($r=-0.37$, $n=34$, $P<0.05$). A similar situation was observed between TNF-α and venous pH ($r=-0.4$, $n=42$, $P<0.01$). We found no statistically significant relationship between plasma TNF-α levels and the age of the patients, renal creatinine clearance, plasma albumin, triglycerides, AT III, fibronectin, vitamin B_{12}, folic acid, P, K, creatinine or ferritin.

Cholecystokinin (CCK) plasma levels

High plasma CCK levels were found in 38 of 45 patients, mean 45.94 $\pm$ 32.28 (3.8–131.5 pg/ml)

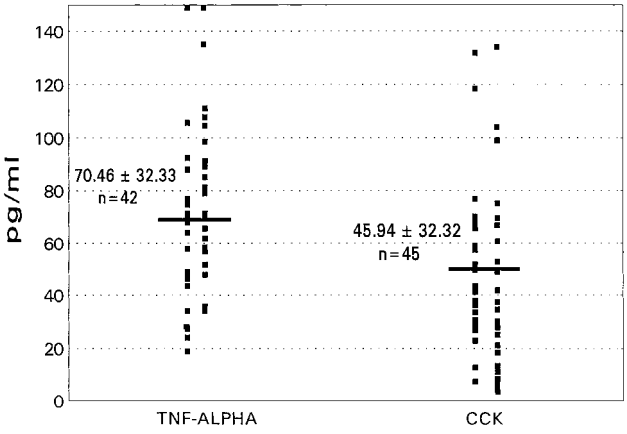

Fig. 1. TNF-α and cholecystokinin (CCK) mean and individual plasma levels in this series. Normal range for TNF-α and CCK, 3–20 and 12–20 pg/ml, respectively.

Table 2. TNF-α plasma levels and nutritional markers

Marker	TNF-α (pg/ml)	Mean ± SD	*n*	*P*
RBP	<65	13 ± 4.67	13	<0.05
(mg/dl)	⩾65	9.6 ± 2.6	21	
Transferrin	<65	268 ± 52.3	20	<0.05
(mg/dl)	⩾65	236.4 ± 40.1	22	
Iron	<65	70.8 ± 25.5	20	<0.05
(μg/dl)	⩾65	54.9 ± 18.9	22	
Cholesterol	<65	242.4 ± 44.4	20	<0.05
(mg/dl)	⩾65	213.6 ± 41.8	22	
Blood urea	<65	158.6 ± 32.7	20	<0.05
(mg/dl)	⩾65	131.7 ± 38.3	22	
CCK	<65	55.5 ± 34.1	20	<0.05
(pg/ml)	⩾65	36.4 ± 30	22	
Prealbumin				
<30 (mg/dl)	91.7 ± 35.8		13	<0.05
⩾30	62.9 ± 27.9		22	
BMI[a]				
<30 (kg/m^2)	76.5 ± 35		29	<0.001
⩾30	46.1 ± 10		5	
nPCR (g/kg/day)				
<1.1	79.3 ± 32.6		26	<0.05
⩾1.1	56.1 ± 27		16	
Urea KT/V				
<2.2	78.3 ± 32.9		27	<0.05
⩾2.2	56.3 ± 26.6		15	

[a]Non-diabetic patients.

(Fig. 1). CCK serum levels showed no relationship with CAPD duration.

Anorectic patients showed no significant differences in CCK values compared with asymptomatic patients (46 ± 36.5, $n=15$ vs 42 ± 27.7, $n=15$, n.s.). A similar situation was observed in non-anorectic patients suffering other GI symptoms (50 ± 33.7, $n=15$, vs 42 ± 27.7, $n=15$, n.s.) and in patients with prior diagnoses of acid pylori disease (30.5 ± 23.9, $n=8$, vs 48.4 ± 32.6, $n=33$, n.s.).

Table 3 shows the relationship between CCK plasma levels and nutritional markers. Patients with plasma albumin levels less than 3.9 g/dl showed CCK plasma levels of 39.9 ± 29.4 ($n=25$), whereas patients with plasma albumin levels greater than 3.9 g/dl showed levels of 57.5 ± 34.6 pg/ml ($n=17$, $P<0.05$). Table 2 shows that TNF-α levels greater than 65 pg/ml are associated with significantly lower CCK levels.

We found no statistically significant relationship between CCK and peritoneal glucose load, renal creatinine clearance, cholesterol, AT III, transferrin, serum iron and urea KT/V. However, we found higher CCK levels in patients with KT/V greater than 2.1 (38.6 ± 22.9, $n=23$) compared with those with values less than 2.1 (53.6 ± 39, $n=22$, $P<0.1$, within the limit of statistical significance).

Table 3. Cholecystokinin plasma levels and nutritional markers

Marker	CCK (pg/ml)	Mean ± SD	*n*	*P*
Albumin	<34	3.66 ± 0.5	21	<0.05
(g/dl)	⩾34	3.97 ± 0.4	24	
Fibronectin	<30	38.4 ± 13.6	12	<0.05
(mg/dl)	⩾30	50.1 ± 13.9	22	
Triglycerides	<30	148.5 ± 39.8	17	<0.05
(mg/dl)	⩾30	166.2 ± 65.1	28	
Folic acid	<30	5.6 ± 1.9	17	<0.01
(ng/ml)	⩾30	8.6 ± 4.6	26	
nPCR (g/kg/day)[a]				
<1.1	36.7 ± 28.6		22	<0.05
⩾1.1	56.4 ± 34.3		14	
α-1-antitrypsin				
<350 (mg/dl)	42.6 ± 25.1		32	<0.1
⩾350	102 ± 46.3		4	

[a]For non-diabetic patients.

Neuropeptide Y plasma levels

Lower than normal NPY plasma values were found in 11 patients (22%), normal values in 33 patients (66%) and high values in 6 patients (12%). Patients with anorexia showed lower NPY values (43.2 ± 27.5 pg/ml, $n=14$) than patients without anorexia (64.9 ± 25.5, $n=19$ $P<0.05$). Patients with GI symptoms also showed lower values (42.8 ± 25.3, $n=17$) than those without GI symptoms (64.9 ± 25.5, $n=19$, $P<0.05$). Patients with prior GI disease showed no significant differences with respect to non-diagnosed patients.

Table 4 shows the relationship between NPY values and nutritional markers. A direct linear relationship appeared between NPY and RBP levels ($r=0.27$, $n=37$, $P<0.05$). The six patients (one diabetic) with NPY values greater than 80 pg/ml had a shorter PD term (9 ± 10.52 months) than the patients with NPY less than 80 pg/ml (34.1 ± 36.7 months, $n=44$, $P<0.01$: nine were diabetics). These differences were maintained for non-diabetic patients.

The NPY plasma levels had a significant inverse correlation with plasma TNF-α levels ($r=-0.42$, $n=41$, $P<0.01$, Figure 2). The mean plasma TNF-α level was also significantly lower in patients with NPY levels greater than 80 pg/ml (33.5 ± 8.9, $n=4$) compared with patients with levels lower than 80 pg/ml (74.4 ± 31.9, $n=37$, $P<0.001$). We found no association between

Table 4. Neuropeptide Y plasma levels and nutritional markers

Marker	NPY (pg/ml)	Mean ± SD	*n*	*P*
Transferrin	<50	238.4 ± 46.9	23	<0.05
(mg/dl)	⩾50	268.4 ± 48.6	27	
Prealbumin	<50	31.1 ± 10	23	<0.05
(mg/dl)	⩾50	37 ± 6.9	18	
RBP	<50	9.8 ± 3.3	20	<0.05
(mg/dl)	⩾50	12.6 ± 4	17	
nPCR	<50	0.96 ± 0.17	23	<0.05
(g/kg/day)	⩾50	1.11 ± 0.2	27	
KT/V	<45	2.03 ± 0.24	18	<0.05
	⩾45	2.34 ± 0.57	32	
BMI (kg/m^2)[a]				
<30	46.6 ± 27.1		32	<0.05
⩾30	70.6 ± 23.4		8	

[a]Non-diabetic patients.

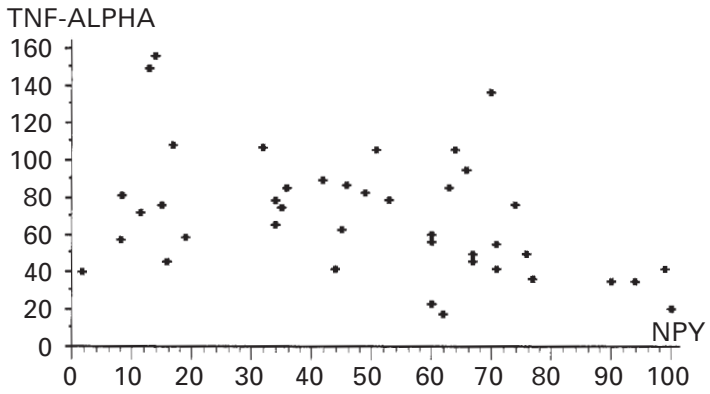

Fig. 2. Linear regression analysis: significant inverse correlation between TNF-α and neuropeptide Y plasma levels ($r = -0.41$, $n = 41$, $P < 0.01$).

NPY values and renal creatinine clearance, plasma albumin, CCK, AT III, fibronectin, ceruloplasmin, α-1-antitrypsin, cholesterol, triglycerides, P, K, BUN, glucose concentration in dialysate and peritoneal ultrafiltration capacity.

Finally, Table 5 shows a summary of nutritional and dialysis parameters in anorectic and asymptomatic patients. Notable statistically significant differences are found in age, PD duration (months), serum albumin, AT-III, TNF-α, NPY, nPCR and urea KT/V, this last only the case of non-diabetic patients.

Discussion

Anorexia is one of the most frequent symptoms in dialysis patients. Several factors have been related to anorexia: middle molecular weight molecule retention [7,26,27], uremic toxicity [28], metabolic and biochemical disorders and abnormalities in cell composition and metabolism [20]. The results of the present study concur with the idea that several plasma substances (TNF-α, cholecystokinin and neuropeptide Y), related to the hunger–anorexia cycle, might also be of importance in PD patients. This is suggested by direct and indirect relationships between nutritional parameters and the circulating levels of these substances.

TNF-α, is responsible for cachectic and anorectic effects in wasting syndrome [9–12,29]. Under experimental conditions, TNF-α administered by the peripheral or intracerebral route decreases food intake through effects on the hunger center [29,30]. Nine fragments have been isolated, each with different actions. The 69–100 fragment has suppressive effects on food intake [30].

We found high TNF-α plasma levels in 97.6% of our patients. Patients with anorexia showed higher levels than those without. Several reports [13,31] have found increased TNF-α plasma levels in non-dialyzed and PD patients. However, McKenna *et al.* [32] did not find high TNF-α production in cultured CAPD patient mononuclear cells. Methodological differences (plasma levels instead of biological activity) may in part explain these discrepancies. TNF-α increased synthesis by cells damaged by hypertension, atherosclerosis, cardiomyopathy [33], liver diseases [34] and uremia [13,14,31] have been shown. Chronic elevated TNF-α levels are more important than acute increments for anorexia induction, this is likely to be due to the short TNF-α half-life (27 min). Plasma concentration peaks are less

Table 5. Differences between anorectic and asymptomatic patients

Parameter	Anorectic		Asymptomatic		
	Mean ± SD	*n*	Mean ± SD	*n*	*P*
Age (years)	55.1 ± 15.9	16	45 ± 13.7	20	< 0.05
PD duration (months)	42.2 ± 30.8	16	15.4 ± 27.8	20	< 0.01
Phosphorus (mg/dl)	4.8 ± 1.3	16	5.7 ± 1.5	20	< 0.1
Albumin (g/dl)	3.5 ± 0.6	16	3.9 ± 0.3	20	< 0.05
Prealbumin (mg/dl)	28.2 ± 13.3	13	35.2 ± 5.8	15	< 0.1
Antithrombin-III (%)	89.5 ± 14.7	11	104.7 ± 12.5	17	< 0.01
TNF-α (pg/ml)	75.9 ± 34	15	52.1 ± 24.5	13	< 0.05
NPY (pg/ml)	43.2 ± 27.4	14	64.9 ± 25.5	19	< 0.05
nPCR (g/kg/day)	0.91 ± 0.15	16	1.06 ± 0.2	20	< 0.05
Urea KT/V	2 ± 0.32	16	2.3 ± 0.54	20	< 0.1
Urea KT/V[a]	1.91 ± 0.24	12	2.37 ± 0.55	17	< 0.01
Cr. Clearance (ml/min)	0.78 ± 1.2	16	3.29 ± 2.2	20	< 0.001

[a]Non-diabetic patients.

important than stable brain concentrations. Chronic administration of low doses of recombinant-murine-TNF-α causes persistent anorexia [35]. TNF-α might operate in this way in PD patients.

Long-term PD patients showed higher TNF-α plasma levels. This relationship has been found in HD patients by Herbelin *et al.* [14]. Although PD patients do not suffer stimulation by HD membranes, a decrease in renal clearance and an increase of synthesis by other conditions [13,14,31–34,36] may explain these data. Our results show that lower TNF-α values are associated with urea KT/V values greater than 2.2, whereas no relationship with residual renal function is demonstrated, suggesting that dialysis efficacy plays a role in TNF-α elimination.

Another TNF-α source is GI tract [37,38]. Our patients with prior acid pylori disease had higher TNF-α plasma levels. TNF-α may be produced by intestinal cells in inflammatory bowel disease [37] and anti-TNF-α monoclonal antibodies have been used for relapse treatment in Chron's disease [38]. Patients with pyloric ulcer associated with *Helicobacter pylori* infection have high TNF-α, IL-1-β and IL-8 production by antral mucosa cells [39].

If we recognize an anorexigenic effect of TNF-α in PD patients, we should demonstrate that the high levels found influence nutritional markers. The negative correlation between TNF-α and short-half life plasma proteins rules out the existence of an increase in acute phase reactants. Plasma albumin, transferrin, cholesterol and creatinine are representative of long-term nutritional parameters. TNF-α showed a negative relationship with transferrin and cholesterol; transferrin is a marker of energy status and low levels of cholesterol have been related to mortality [1,2]. However, we found a non-significant tendency in the relationship between TNF-α and plasma albumin. TNF-α inhibits hepatic albumin synthesis in rats, possibly by direct inhibition of gene expression [40]. Peritoneal or renal protein losses and the slower albumin synthesis rate than that of prealbumin or RBP, may modify this relationship.

Prealbumin, RBP, AT III, fibronectin, ceruloplasmin and lymphocyte count are medium-term nutritional markers. There was a statistically significant negative linear correlation between TNF-α plasma levels and RBP. TNF-α values also showed an inverse association with prealbumin levels greater and lower than 30 mg/dl. Prealbumin and RBP have proved useful in the recognition of moderate and severe malnutrition in PD patients [6].

We also found a negative association between TNF-α levels and early food intake markers (nPCR and urea nitrogen). An important factor in the genesis of dialysis anorexia is underdialysis. Several authors have observed transitory improvements of appetite after increasing dialysis dosage [41,42]. According to our data, lower urea KT/V values are associated with anorexia in non-diabetics and high TNF-α plasma levels (Tables 2 and 5).

Obese patients showed lower TNF-α levels than non-obese patients ($BMI < 30\ kg/m^2$). A similar observation was found by Chollet-Martin *et al.* [43]. A direct lipolytic effect of TNF-α has been demonstrated [29]. TNF-α induced an increase in serum triglycerides over a 10-day period [29], but no significant relation between these parameters was found ($r = 0.22$, n.s.).

Another factor involved in dialysis malnutrition is acidosis [44]. Metabolic acidosis is an effect of R-m-TNF-α long-term administration [29,35]. The inverse correlation between TNF-α and blood pH ($r = -0.4$, $n = 42$, $P < 0.01$) agrees with this finding. Acidosis increases muscle proteolysis in rats via the ubiquitin–proteasome pathway [45]. Other conditions associated with inflammation, and presumably high TNF-α values, exhibit catabolism by activating the same system of protein degradation as acidosis [46]. We cannot rule out that high TNF-α plasma levels could represent a chronic inflammatory state as it has been found in haemodialysis, although not in PD patients, to be related with malnutrition [32,47]. TNF-α seems therefore to contribute to acidosis and malnutrition in PD patients.

CCK is a GI peptide released by duodenal cells in the intestinal lumen in response to protein, fat, acid and calcium intakes. It stimulates gall bladder contraction and pancreatic enzyme secretion and inhibits gastric emptying. Through peripheral and brain receptors, CCK causes anorexia by a satiety effect [15–19]. High CCK plasma levels were found in 84% of our patients. The decrease in CCK-33 the fragment renal clearance [21,23] and its longer half-life, 10× that of CCK-8, may explain this feature [22]. In our patients, anorexia showed no relationship with CCK plasma levels. The influence of other factors on CCK release, such as peritoneal glucose absorption could explain this apparent contradiction. Plasma glucose is a negative factor in the feedback of CCK release by exocrine pancreatic stimulation (trypsin) [48]. In addition, α-1-glycoprotein composed of hexoses such as glucose, mannose or galactose, and amino acids have a satiety effect *per se* [20]. Some studies of anorexia nervosa indicate that peak CCK plasma levels, stimulated by food intake, occurred earlier than in normal subjects (30 min vs 60 min). This finding suggests that high initial CCK plasma rise may contribute to the abnormal perception of satiety [49]. Although we did not investigate the CCK cycle, the high levels of plasma CCK and the continuous peritoneal glucose absorption could induce a loss of the normal CCK–glucose feedback function in PD patients.

With respect to CCK and nutritional markers, lower CCK plasma levels were associated with malnutrition data. The difference was observed with CCK values lower and higher than 30 pg/ml. Plasma albumin showed a direct association with CCK levels; levels greater than 3.9 g/dl were associated with CCK levels greater than 30 pg/ml. However, as albumin binds CCK, this association could be explained [50]. The remaining nutritional parameters (Table 3) were also positively correlated with CCK. These features may

indicate that CCK plasma levels are a food intake marker in PD patients.

Neuropeptide Y (NPY) belongs to the pancreatic polypeptide family and is involved in drinking and eating [51,52] control and in intestinal motility and secretion [48]. It is the most potent orexigen known to be related to the pathogenesis of obesity [24].

Thirty-four percent of our patients showed abnormal NPY plasma values (22% lower and 12% higher than normal). In uremic patients, the state of NPY plasma levels is poorly known. Factors related to NPY release were excluded in our population (inadequate hydration state, no fasting or insulin use, which was postponed to after sampling in diabetics) [25]. NPY values were not related to peritoneal ultrafiltration capacity or glucose concentration in PD fluid. An increase in NPY plasma levels has also been reported during hemodialysis sessions associated with fluid removal [52] but this factor did not contribute in our patients. Since several animal models have suggested that the splanchnic area and kidney are important sources of NPY [53,54], we cannot rule out that different nephropathies or renal functions explain these differences.

A negative linear correlation was found between NPY and TNF-α (Figure 2). To our knowledge, this has not been described previously and could be another way by which TNF-α causes anorexia. There are several studies demonstrating that cytokines produce stimulation of GI peptide release. For instance, IL-1 induces insulin secretion [55] and contributes to the anorexigenic effect of CCK [56]. TNF-α increases serum growth hormone levels and catabolic stress hormone release [57]. We suggest that TNF-α could contribute to lower NPY levels in anorectic PD patients. The powerful orexigen effect of NPY injected into the hypothalamic region is well known [58]. We have found a positive relationship between nutritional markers and NPY. Non-diabetic obese patients showed high NPY plasma levels. NPY has been implicated in body weight regulation [59] and lipolysis inhibition in human fat cells [60]; NPY inhibition has been demonstrated by the obese gene product, leptin [24]. NPY may improve nutritional status by increasing food intake and through its metabolic effects, such as reduction of glycogenolysis and stimulation of gluconeogenesis [59].

In conclusion, our data suggest that high TNF-α and low NPY serum levels are associated with anorexia. High serum TNF-α levels might be responsible for uremic anorexia in PD patients. High TNF-α, low CCK and low NPY serum levels are also related to poor nutritional status. Further research on these endogenous substances is required.

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Midpeliq Reduces Glucose Load

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The rate of technique failure is still high in Japan for peritoneal dialysis (PD) patients. Of the dropouts who have been treated with PD for more than 6 years, about half suffer from ultrafiltration failure. That condition is supposedly related to the bioincompatible aspects of conventional acidic PD solutions.

In 2001, a neutral-pH, lactate-buffered PD solution with low glucose degradation products (GDPs), Midpeliq (Terumo Corporation, Tokyo, Japan), was developed and began to be used in Japan. After switching 3 patients from conventional acidic PD solution to Midpeliq, we observed that 2 patients could then use lower-glucose PD solutions.

Case 1 was a 42-year-old woman with a 10-year history of PD. In February 2001, she was switched from Peritoliq (Terumo) to Midpeliq. One month later, she complained of dizziness, and her blood pressure was found to be down to 96/60 mmHg. Post-change fluid removal increased to 1,481 mL from 1,238 mL ($p < 0.02$). Before the solution switch, this patient exchanged 4 times daily, using 2 L of 2.5% Peritoliq each time. From 3 months after the solution switch, she exchanged 3 times daily using 2 L of 2.5% Midpeliq and 1 time daily using 2 L of 1.35% Midpeliq. Fluid volume removal stayed almost the same.

Case 2 was a 52-year-old man with a 9-year history of PD. In June 2002, he was switched from Dianeal 4 (Baxter Healthcare, Tokyo, Japan) to Midpeliq. After the change, his daily drainage volume increased from approximately 1,500 mL to 2,000 mL. He began to use 2 L of 1.35% Midpeliq 4 times daily instead of 2 L of 1.5% Dianeal 3 times daily and 2 L of 2.5% Dianeal 1 time daily. At 1 month after the solution switch, his drainage volume was still approximately 1,500 mL daily.

Our observations suggest that new, neutral-pH PD solutions such as Midpeliq might reduce the glucose load in addition to having low GDPs and fewer toxic effects on the peritoneum.

From: Nikko Memorial Hospital, Hokkaido, Japan.

Key words
Glucose load, neutral peritoneal dialysis solution

Introduction

The rate of technique failure is still high in Japan for peritoneal dialysis (PD) patients. Of the dropouts who have been treated with PD for more than 6 years, about half suffer from ultrafiltration failure (1). That condition is supposedly related to the bioincompatible aspects of conventional acidic PD solutions. Aspects of PD solutions that have been considered responsible for bioincompatibility are low pH, high glucose concentration, high osmolality, high lactate concentration, and presence of glucose degradation products (GDPs). To correct those drawbacks, a neutral-pH, lactate-buffered, double-chamber technology with a reduced amount of GDPs, Midpeliq (Terumo Corporation, Tokyo, Japan; Figure 1), was developed in 2001 and began to be used in Japan. In many trials and experiences, neutral-pH, lactate-buffered, low-GDP PD solutions—including Midpeliq—have been reported to have beneficial effects on PD patients.

Given the low pH and high GDPs of many PD solutions, much concern has developed regarding glucotoxicity on the peritoneal membrane, because glucose is supposed to be the principal cause of peritoneal membrane damage in long-term PD patients (2). To reduce the deleterious effects of glucose load on the peritoneal membrane, the use of non glucose-based PD solutions has been proposed (3). Little attention has been drawn to the effects of neutral-pH, lactate-buffered, glucose-based PD solutions with low GDPs (such as Midpeliq) on reducing the glucose load. After switching 3 patients from conventional acidic PD solution to Midpeliq, we observed 2 patients who were able to use lower-glucose PD solutions. In the present article, we want to emphasize the reduction in glucose load offered by neutral-pH solutions, so that that aspect of treatment won't be forgotten. In Japan, production of conventional acidic PD solutions has been discontinued. Those solutions are being replaced by neutral-pH, lactate-buffered, glucose-based PD

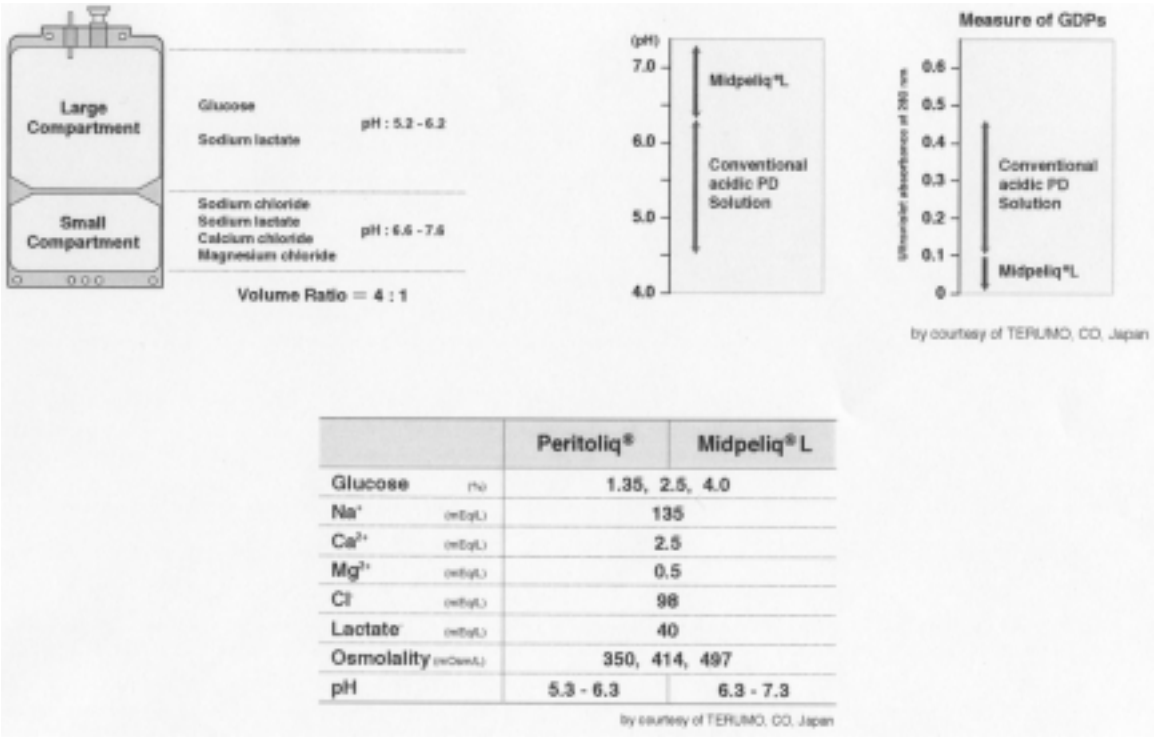

FIGURE 1 Composition of, and levels of glucose degradation products (GDPs) in, Midpeliq.

solutions, and, as a result, studies like ours will cease to appear.

Patients and methods

Case 1

Case 1 was a 42-year-old woman with a 10-year history of PD. In February 2001, she was switched from Peritoliq (Terumo) to Midpeliq. One month later, this patient complained of dizziness. Her blood pressure had dropped to 90/60 mmHg. The symptoms were thought to be related to dehydration, caused by too much fluid removal. She recovered after fluid replacement. After the solution switch, her fluid removal had actually increased to 1,481 mL from 1,238 mL ($p < 0.001$). When her body weight decreased, she was advised to use reduced-glucose PD solutions. From 3 months after the switch, she exchanged 3 times daily using 2 L of 0.5% Midpeliq and 1 time daily using 2 L of 1.35% Midpeliq. Her fluid removal remained at about the same volume as that seen when she exchanged 4 times daily using 2 L of 2.5% glucose Midpeliq (Figure 2). Blood chemistry showed no significant change, including no change in serum albumin concentration. The 4-hour dialysate-to-plasma creatinine (D/P Cr) at 1 month before the switch was 0.55; at 6 months after the switch, it was 0.59.

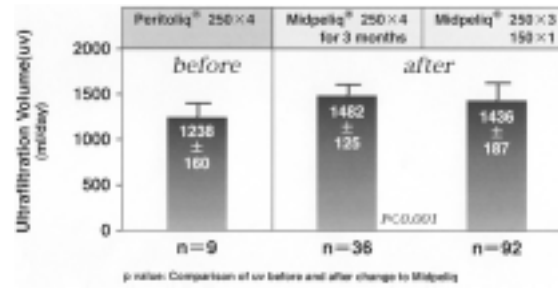

FIGURE 2 Ultrafiltration volumes before and after a switch to Midpeliq in case 1.

Case 2

Case 2 was a 52-year-old man with a 9-year history of PD. In June 2002, he was switched from Dianeal PD-4 (Baxter Healthcare, Tokyo, Japan) to Midpeliq. After the change, his daily drainage volume increased significantly to 2,037 mL from 1,550 mL. He began to use 2 L of 1.35% Midpeliq 4 times daily instead of 2 L of 1.5% Midpeliq 3 times daily and of 2 L of 2.5% Midpeliq 1 time daily (Figure 3). At 1 month after the solution switch, his daily drainage volume was still approximately 1,640 mL. Blood chemistry showed no significant change, although a downward trend in serum total cholesterol and serum triglycerides was noted. His 4-hour D/P Cr at 2 months before the switch was 0.49; 4 months after the switch, it was 0.51.

Discussion

Neutral-pH, lactate-buffered, and reduced-GDP PD solution was developed to prevent PD dropout owing to ultrafiltration failure. More patients on PD using the novel neutral solution are expected to continue PD longer than before. The less deleterious effects of the new solution on the peritoneal membrane are thought to be related to neutral pH and low concentrations of GDPs. Although similar observations have not been reported previously, our observations suggest that the reduction in glucose seen with neutral-pH, lactate-buffered, glucose-based PD solutions with low GDPs (such as Midpeliq) might have additional effects on keeping peritoneal membrane function stable.

Following the correction of acidic pH and the presence of GDPs in PD solutions, more attention has focused on the glucotoxicity of PD solution for the peritoneal membrane (2). Holmes and Shockley (3) proposed to reduce the deleterious effects of glucose load on the peritoneal membrane by using non glucose-based PD solutions. Davies *et al.* (4) observed that higher peritoneal glucose exposure caused changes in peritoneal solute transport with time on PD. That finding suggested that using reduced-glucose PD solution would make it possible to prolong peritoneal membrane structure and function.

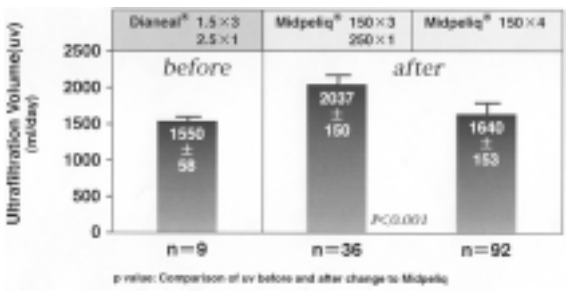

FIGURE 3 Ultrafiltration volumes before and after a switch to Midpeliq in case 2.

Our observations suggest that a reduction in glucose load is made possible by the increased ultrafiltration volume seen after a change to Midpeliq. The reason why Midpeliq resulted in increased ultrafiltration volume as compared with conventional acidic PD solutions is still unclear. The question of whether new neutral-pH PD solutions cause increased ultrafiltration volume after a change from conventional acidic PD solutions is still not settled. Coles *et al.* (5) did not confirm that the use of neutral-pH fluid was associated with increased ultrafiltration. On the other hand, Tranæus (6) noticed that ultrafiltration increased significantly from baseline (150 mL for the total 6-month treatment period) in patients using bicarbonate/lactate–based PD solution. We also observed an increment in ultrafiltration after a switch to Midpeliq in 2 patients. However, Tranæus did not report that the new, neutral-pH PD solutions lead to a reduced glucose load owing to the use of lower-glucose PD solutions. That difference between our observations and his might be attributable to the use of a different, neutral PD solution or to other, unknown factors. We need further study to clarify the reasons for the increase in ultrafiltration and the ability to reduce the glucose load after a change from conventional acidic PD solution to Midpeliq.

Conclusions

Our observations suggest that new, neutral-pH PD solutions such as Midpeliq might enable a reduction in glucose load, in addition to having lower GDPs and fewer toxic effects on the peritoneum.

Addendum

After a change to Midpeliq, a 3rd patient showed increased drainage volume, but no decrease in glucose load. At the time of writing (starting from December 2002), that patient has been using 2 L of 1.35% Midpeliq 3 times daily and 2 L of 2.5% Midpeliq 1 time daily instead of 2 L of 1.35% Midpeliq 2 times daily and 2 L of 2.5% Midpeliq 2 times daily.

Acknowledgment

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Malnutrition–Inflammation Syndrome Is Associated with Endothelial Dysfunction in Peritoneal Dialysis Patients

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Endothelial dysfunction with atherosclerosis is a recognized complication of uremic patients. The hypoalbuminemia of peritoneal dialysis (PD) patients can induce a hypercoagulable and atherogenic state. In this study, we investigated the role played by malnutrition–inflammation syndrome on endothelial function markers in PD patients.

We measured markers of nutrition [normalized protein catabolic rate (nPCR), albumin, prealbumin, insulin-like growth factor 1 (IGF-1), transferrin, and cholesterol], markers of endothelial damage and function [tissue-type plasminogen activator (tPA), thrombomodulin (TM), von Willebrand factor (vWF), and NO_3 (representing NO)], markers of a coagulable state [fibrinogen and plasminogen activator inhibitor 1 (PAI-1)], markers of inflammation [tumor necrosis factor α (TNFα) and C-reactive protein (CRP)], and other endothelial injury factors {lipoprotein(a) [Lp(a)] and homocysteine}. We also performed an endothelial stimulation test consisting of right-arm venous occlusion (VO) for 10 minutes. The patients were divided into four groups according to their clinical atherosclerotic score (CAS).

We studied 45 clinically stable PD patients. At baseline, statistically significant negative linear correlations were found between albumin and age ($r = -0.54$, $p < 0.05$*), albumin and vWF post-VO (*$r = -0.54$, $p < 0.05$*), and albumin and TM (*$r = -0.36$, $p < 0.05$*), which are endothelial damage markers and prothrombotic factors. A positive linear correlation was seen between albumin and NO_3 post-VO (*$r = 0.48$, $p < 0.05$*), indicating a high vasodilatation capacity. C-Reactive protein and TNFα showed a positive linear correlation (*$r = 0.5$, $p < 0.01$*). Similarly, TNFα showed a positive linear correlation with cardiovascular risk markers such as fibrinogen (*$r = 0.79$, $p < 0.01$*), PAI-1 (*$r = 0.44$, $p < 0.05$*), and homocysteine (*$r = 0.37$, $p < 0.05$*). Creatinine clearance showed a negative linear correlation with TM (*$r = -0.36$, $p < 0.05$*). Patients with albumin < 4 g/dL showed a lower tPA ratio, lower NO_3, and a higher CRP, TNFα, and Lp(a) than did patients with albumin > 4 g/dL [tPA ratio: 2.1 ± 1.56 (*$n = 29$*) vs. 2.6 ± 2.3 (*$n = 16$*), $p < 0.05$; NO_3: 47 ± 27 μg/mL vs. 69 ± 33 μg/mL, $p < 0.05$; CRP: 1.8 ± 3 mg/dL vs. 1.1 ± 1.6 mg/dL, $p < 0.05$; TNFα: 44.4 ± 16 pg/mL vs. 36.6 ± 21.4 pg/mL, $p < 0.05$; Lp(a): 55 ± 39 mg/dL vs. 33 ± 21 mg/dL, $p < 0.05$]. Patients with a worse CAS showed higher homocysteine levels and lower albumin values. Those relationships were maintained in both periods of the study. We found no relationships between dialysis dose and endothelial function markers. In conclusion, malnutrition–inflammation syndrome may contribute to endothelial dysfunction and, consequently, to prothrombotic and proatherogenic processes in PD patients.*

Key words
Inflammation, malnutrition, endothelial dysfunction, cardiovascular risk

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Introduction

Malnutrition and cardiovascular complications are the main causes of morbidity and mortality in dialysis patients (1). Recently, both processes have been suggested to possibly be part of one large, complex pathway: malnutrition, inflammation, and atherosclerosis (MIA) syndrome (2). The syndrome adds to the traditional cardiovascular (CV) risk factors (hypertension,

dyslipidemia, smoking, obesity, and sedentary lifestyle), with inflammation playing a crucial part in MIA pathogenesis.

Endothelial dysfunction is possibly the starting and common element of early atherosclerosis (3). It also is a prominent feature of uremic status (4), and owing to protein malnutrition, it may induce procoagulant events (5). Procoagulant status is recognized as being proatherosclerotic (3–5).

Recently, a strong relationship has been described between inflammation and endothelial dysfunction in renal (6) and non renal patients (7). Experimentally, intermittent inflammatory episodes have been suggested to induce impairment of endothelium-dependent vascular relaxation (8). Moreover, an epidemiologic association between C-reactive protein (CRP) and CV events has been accepted (7). Important support for the inflammation hypothesis has been provided by the association between endothelial dysfunction and chronic, silent infection by *Chlamydia pneumoniae,* several viruses, or *Helicobacter pylori* (9). Furthermore, we found an association between *Helicobacter pylori* infection and malnutrition through anorexia in peritoneal dialysis (PD) patients (10). The eradication of *Helicobacter pylori* reduced plasma levels of cytokines [interleukin 1 (IL-1) and tumor necrosis factor α (TNFα)] and improved nutrition status.

Our aim was to establish the association between endothelial dysfunction, malnutrition, and inflammation, considering that endothelial dysfunction is the initial phenomenon in the atherosclerosis pathway.

Patients and methods

We studied 45 clinically stable PD patients, 20 men and 25 women with an age range of 25 – 86 years (mean: 55 ± 13.2 years). The mean period on PD was 29.3 ± 28 months (range: 1 – 120 months). No active disorders were present during the 3 months before the study. Patients with recognized endothelial and active systemic diseases, and those with immunosuppression, were excluded. We also excluded patients treated with recombinant human erythropoietin, owing to the potential effect of that drug on endothelium (11).

In the 45 patients, the causes of renal failure were diabetes (n = 11), nephrosclerosis (n = 7), glomerulonephritis (n = 6), polycystic kidney disease (n = 6), tubulointerstitial disease (n = 6), unknown (n = 6), systemic disease (n = 2), and congenital disease (n = 1). Thirty-eight patients had been diagnosed with hypertension. For medical treatment, 7 patients were receiving angiotensin converting-enzyme inhibitors; 4 were using calcium channel blockers; 7 were using other drugs; and 15 were receiving various drug combinations. Hypertension was controlled by ultrafiltration in 5 patients. By echocardiogram, we found 17 patients with mild left ventricular hypertrophy (LVH), 16 with moderate LVH, and 5 with severe LVH (Penn convention). No LVH was apparent in 7 patients. Eight patients used acetylsalicylic acid, and three, pentoxifylline. Five patients were active smokers; 13 had a past history of smoking; and 27 had never smoked.

We measured these parameters:

- Dialysis adequacy, by weekly Kt/V urea and normalized equivalent of protein nitrogen appearance (nPNA)
- Nutrition, by the markers albumin, by the colorimetric method; cholesterol (Hitachi 704: Boehringer Mannheim, Mannheim, Germany); transferrin, prealbumin, and retinol binding protein, by the immunonephelometric method (Behring Nephelometer Terminal SA: Behringwerte AG, Marbus, Germany); and insulin-like growth factor 1 (IGF-1), by radioimmunoassay after acid–ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, U.S.A.). Maximal intra-assay and inter-assay coefficients of variation were 2.9% and 11.4%, respectively. The IGF-1 sensitivity was 12.9 ng/mL.
- Inflammation, by the markers CRP {by the immunonephelometric method [ELISA (Vectastin: Vector Laboratories, Burlingame, CA, U.S.A.)]} and TNFα (Easia: Medgenix Diagnostics SA, Fleurus, Belgium)
- Endothelial function, by venous occlusion test (VOT), an endothelial stimulation test consisting of a right-arm venous occlusion {stasis was achieved for 10 minutes by applying a sphygmomanometer cuff inflated to a pressure midway between the systolic and diastolic values [mean arterial pressure = (systolic pressure + diastolic pressure) / 2]}; by endothelial factors associated with endothelial fibrinolytic capacity [tissue-type plasminogen activator (tPA)] pre-VOT and post-VOT; by endothelial damage markers [von

Willebrand factor (vWF), thrombomodulin (TM)]; and by relaxation factor NO (measured as NO_3 concentration) pre-VOT and post-VOT (12)
- Coagulation and CV risk status, by the markers fibrinogen [inflammatory and procoagulant marker (by thrombin time method as described by Clauss)], lipoprotein(a) {Lp(a) [by sandwich-type enzyme-linked immunoassay (TintElize: Biopool, Umea, Sweden)]}, and homocysteine (by high-pressure liquid chromatography)

Blood samples were drawn in resting and fasting conditions, between 9:00 h and 11:00 h, and were collected into Vacutainer tubes (Becton–Dickinson, Mountain View, CA, U.S.A.) containing 0.129 mol/L sodium citrate. The samples were centrifuged and tested immediately or stored at –70°C until assayed. Plasma antigenic to tPA was determined by ELISA (Coaliza: Chromogenix AB, Mölndal, Sweden); vWF by Asserachrom (Boehringer Mannheim, Meylan, France); TM by Asserachrom (Diagnostica Stago, Asnières, France); and NO_3 by capillary electrophoresis (11). A clinical atherosclerotic score (CAS: I – IV) was applied to all patients, defining the highest risk as CAS IV (13). Of the 45 patients, 14 were CAS I, 17 were CAS II, 4 were CAS III, and 10 were CAS IV.

Statistical analysis

Results are given as means and ranges. Comparisons between groups were performed using the Mann–Whitney rank sum *U*-test. Also, we used the Student *t*-test for paired and unpaired data.

TABLE I Overall data

Parameter	*Mean±SD*	*Normal range*
Hemoglobin (g/dL)	10.3±1.9	10–12
Systolic BP (mmHg)	138±17.7	<135
Diastolic BP (mmHg)	74±9.2	<85
CRP (mg/dL)	1.34±2.15	0.5
TNFα (pg/mL)	38.12±20	<20
Creatinine (mg/dL)	9.73±2.7	Variable[a]
Albumin (g/dL)	3.73±0.47	>4
Prealbumin (mg/dL)	31.4±11.3	>30
IGF-1 (ng/mL)	254±155.5	54–450
Transferrin (mg/dL)	259.8±58	210–390
Cholesterol (mg/dL)	212.5±38.8	150–220
Weekly Kt/V urea	2.21±0.46	>2
Daily nPNA (g/kg)	1.06±0.27	>1.1
PAI-1 (ng/mL)	9.9±7.41	<10
tPA pre-VOT (ng/mL)	8±4.15	1–12
tPA post-VOT (ng/mL)	17.32±11.2	Variable
tPA ratio	2.16±2.7	>2
vWF pre-VOT (%)	221.6±45.6	60–150
TM pre-VOT (mg/dL)	292.9±108.9	14–55
NO_3 pre-VOT (μmol/L)	53.9±30.7	40–60
NO_3 post-VOT (μmol/L)	54±30.4	Variable
NO_3 ratio	0.99±1	Variable
Fibrinogen (mg/dL)	468±106.6	150–350
Lipoprotein(a) (mg/dL)	47.6±35.4	<20
Homocysteine (mmol/L)	37.6±11	5–10

[a] For dialysis patients.
SD = standard deviation; BP = blood pressure; CRP = C-reactive protein; TNFα = tumor necrosis factor α; IGF-1 = insulin-like growth factor type 1; nPNA = normalized equivalent of protein nitrogen appearance; PAI-1 = plasminogen activation inhibitor type 1; tPA = tissue-type plasminogen activator; VOT = venous occlusion test; vWF = von Willebrand factor; TM = thrombomodulin.

Results

Table I shows the overall data for the series. We found high plasma levels of inflammatory markers (CRP, TNFα), low values for markers of nutrition (albumin, prealbumin, IGF-1, nPNA), and high plasma levels of endothelial damage markers (vWF, TM). We also found low plasma levels of fibrinolytic markers (tPA) and high plasma levels of other CV risk markers [Lp(a), homocysteine]. Statistically significant inverse linear correlations were found between albumin and age ($r = –0.54$, $p < 0.05$), albumin and vWF post-VOT ($r = –0.54$, $p < 0.05$), and albumin and TM ($r = –0.36$, $p < 0.05$). Albumin also showed a positive linear correlation with NO_3 post-VOT ($r = 0.48$, $p < 0.05$) and a negative correlation with tPA ratio ($r = –0.33$, $p < 0.05$).

The inflammatory markers CRP and TNFα showed a positive linear correlation ($r = 0.5$, $p < 0.01$). Similarly, TNFα showed a positive linear correlation with CV risk markers such as fibrinogen ($r = 0.79$, $p < 0.01$), PAI-1 ($r = 0.44$, $p < 0.05$), and homocysteine ($r = 0.37$, $p < 0.05$). Finally, TNFα showed a negative relationship with serum albumin ($r = –0.4$, $p < 0.05$).

Table II shows the status of all of those markers according to the malnutrition pattern defined by the Dialysis Outcomes Quality Initiative guidelines (14). Malnourished patients showed elevated markers of inflammation and of endothelial dysfunction as compared with patients whose albumin was higher than 4 g/dL. Those differences were not due to differences in residual renal function, age, arterial pressure, time

TABLE II Markers of malnutrition–inflammation syndrome and of endothelial function

Parameter	*Albumin (<4 g/dL)* *(*n*=29)*	*Albumin (⊕4 g/dL)* *(*n*=16)*	p *Value*
CRP (mg/dL)	1.8±3	1.1±1.6	<0.05
TNFα (pg/mL)	44.4±16	36.6±21.4	<0.05
Age (years)	59.4±12.48	51.2±11.6	0.054 (NS)
Time on PD (months)	27.4±30.7	35±28.1	NS
Weekly Kt/V urea	2.29±0.55	2.13±0.27	NS
Daily nPNA (mg/kg)	1.3±0.2	1.42±0.4	0.06 (NS)
CCr (mg/dL)	1.9±2	1.67±2.17	NS
Prealbumin (mg/dL)	27.8±9.2	41±11.3	<0.001
PAI-1 (ng/mL)	8.7±3.8	12.6±12	NS
tPA ratio	2.1±1.56	2.6±2.3	<0.05
NO_3 post-VOT (μmol/L)	46.9±27	68.9±33	<0.05
vWF pre-VOT (%)	245.2±77	220±33	0.061 (NS)
Lipoprotein(a) (mg/dL)	54.7±39	32.7±20.6	<0.05
Fibrinogen (mg/dL)	469.4±136	467±209	NS
Homocysteine (mmol/L)	37.3±12.5	38.4±7.7	NS
TM pre-VOT (mg/dL)	281.8±104	316±119.4	NS
CAS (I/II/III/IV)	(7/13[a]/3/6)	(7/4[a]/1/4)	[a] <0.05

CRP = C-reactive protein; TNFα = tumor necrosis factor α; PD = peritoneal dialysis; nPNA = normalized equivalent of protein nitrogen appearance; CCr = creatinine clearance; PAI-1 = plasminogen activation inhibitor type 1; tPA = tissue-type plasminogen activator; VOT = venous occlusion test; vWF = von Willebrand factor; TM = thrombomodulin; CAS = clinical atherosclerotic score.

on dialysis, or adequacy parameters. However, CAS was a distinguishing parameter, revealing that higher values were associated with other CV risk factors such as homocysteine level: CAS I versus CAS II (32.9 ± 8.2 mmol/L vs. 39.9 ± 12.5 mmol/L, $p < 0.05$), CAS II versus CAS IV (39.9 ± 12.5 mmol/L vs. 43.2 ± 13.7 mmol/L, $p < 0.05$), and CAS III versus CAS IV [42.4 ± 13.5 mmol/L vs. 43.2 ± 13.7 mmol/L, p = nonsignificant (NS)].

Discussion

Endothelium is an active barrier between vessel and bloodstream with several important roles, but its location makes evaluating its functional integrity difficult. Still, several markers have been proposed, including vasoactive dilatation and constriction capacity evaluated by plasma levels of NO and endothelin I, and fibrinolytic capacity estimated by PAI-1 and tPA. Endothelial repair capacity is estimated by cell turnover markers [vWF and TM (11)].

The markers chosen for the present research confirm the existence, in PD patients, of endothelial dysfunction characterized by low fibrinolytic capacity, disorders in endothelial vasoactive function, and elevated plasma markers of endothelial injury (Table I). All of those features were associated with data compatible with inflammatory and malnourished status (Table II).

Endothelial fibrinolytic system

Fibrinolysis is regulated by plasma concentrations of PAI-1 and tPA, which have opposing action. Both regulate plasmin concentration, which activates the coagulation final pathway. In normal conditions, when fibrinolysis is activated, tPA transforms plasminogen into plasmin. Plasmin is degraded in the liver by PAI-1 (5).

The endothelium-derived fibrinolytic glycoprotein tPA is released by exercise, VOT, and stimulation with desmopressin. After stimulation (VOT > 2), tPA values indicate adequate fibrinolytic capacity (4,11,15). Patients with severe atherosclerosis or unstable angina have been demonstrated to show low tPA after stimulation (16). Uremia *per se* is associated with tPA disturbances for unknown causes (4,5,11). Recently, we found that treatment with recombinant human erythropoietin (rHuEPO) in PD patients is associated with a dramatic decrease in tPA post-VOT, theoretically worsening the uremic prothrombotic status (11).

Importantly, in the present research, we found a clear association between elevated pro-inflammatory molecules, malnutrition, and a decrease in tPA. *In vivo*

and *in vitro,* cytokines (IL-1 and TNFα) have been demonstrated to stimulate endothelium, inducing a profound alteration in fibrinolytic capacity mediated by PAI-1 (5,6,17). In the present research, we found no significant differences in PAI-1, indicating that other mechanisms such as alteration in protein hepatic metabolism might be determining PAI-1 levels. In patients with malnutrition and inflammation, liver protein metabolism disorders have been described (15). Relative to tPA, stimulation of cultured human umbilical vein endothelial cells with IL-1 and TNFα reduces tPA synthesis (18), reducing endothelial fibrinolytic capacity. Because tPA can be considered a pro-inflammatory molecule (15), pre-VOT or post-VOT elevation of tPA may result in a reduction in the tPA ratio, inducing procoagulant phenomena.

Endothelial turnover and response to injury

After endothelial damage, vWF is released, stimulating platelet aggregation (11). High levels of vWF have been found in various types of endothelial damage. Von Willebrand factor is considered a true CV risk factor because it promotes thrombosis (5,15,17). In dialysis patients, elevated plasma levels of vWF have been described (11,15,17). Kidneys do not remove vWF; lack of renal excretion therefore does not affect its level in plasma (11). Von Willebrand factor is also an acute-phase reactant; it is increased by cytokines (15). Our results confirm that high TNFα plasma levels are associated with high vWF levels [245.2% ± 77% vs. 220% ± 33%, $p = 0.061$ (NS); Table II]. Definitely, those differences are strong indicators of endothelial damage. In uremia, other factors such as activation of platelets by hemodialysis filters contribute to high vWF levels (15,19).

The structural protein TM is released from the membrane of endothelial cells in injury conditions; its level represents the quantity of dead cells (15,17). Because TM is normally excreted by the kidneys, high plasma levels are found in uremia (11,20). The TM released into plasma activates protein C to inhibit fibrin formation (17). We found no differences in TM plasma levels between patients with and without inflammation. Inflammatory mediators, proteolysis, and oxidation are well known to induce downregulation of TM (5,15). However, in cultured cells, stimulation with high (near-lethal) doses of TNFα induces an increase in TM, representing endothelial injury (5,15). Another potential impact of inflammation on protein C occurs through the loss of protein S function and C4b-binding protein, which worsen procoagulant status (15).

The most potent vasodilator, NO, is synthesized by endothelial cells. It inhibits platelet adhesion, release of mitogenic factors, and proliferation of muscle vessel cells (12). Deficient NO production with early atherosclerosis has been frequently reported (21,22). Administration of recombinant TNFα depressed endothelium-dependent relaxation. Moreover, in cultured endothelial cells, TNFα administration reduced the half-life of the mRNA that encodes for NO synthase (23). We found that the endothelial response to VOT (elevating NO levels) was lower in the group of patients with inflammation and malnutrition (Table II). Another possible explanation for the difference is the low level of arginine in malnourished patients, given that arginine is the substrate to NO synthesis.

Lipoprotein(a) and homocysteine are recognized CV risk factors that are frequently elevated in dialysis patients (5,9). Inflammation causes an increase in the hepatic synthesis of acute-phase reactants. Lipoprotein(a) is one of the proteins that induce a procoagulant status (24). We found no differences between patients in plasma levels of homocysteine (Table II), possibly because inflammation is not a risk factor for homocysteine elevation (5,13,25).

The various *in vivo* and *in vitro* effects associated with TNFα make us think that TNFα is the inductor molecule in endothelial dysfunction. Supporting that idea is the fact that IL-6 infusion in a healthy population is not associated with impaired endothelial function (8). Moreover, infusion of albumin only partially corrects endothelial dysfunction (1). Therefore, we speculate that a sole molecule, TNFα, may be responsible for the multiple parts of MIA syndrome.

Conclusions

The presence of inflammation is associated with malnutrition, endothelial dysfunction, and procoagulant and proatherosclerotic status in PD patients.

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Serum Concentration of Haptoglobin, Adequacy of Peritoneal Dialysis, and Nutrition Status of Patients With and Without Diabetes on Peritoneal Dialysis

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The aim of the present study was to establish a relationship between serum haptoglobin (HTG) concentration, peritoneal dialysis (PD) adequacy, and nutrition status in PD patients with and without diabetes. We measured serum concentrations of HTG, albumin, iron, and cholesterol; platelet count; transferrin saturation (TSAT); weekly Kt/V; and total weekly creatinine clearance (CCr) in 60 patients with and without diabetes who were being treated with continuous ambulatory PD or automated PD. The mean serum HTG concentration in PD patients without diabetes (2.5 ± 1.2 g/L) was elevated and differed significantly from that in PD patients with diabetes (2.0 ± 1.1 g/L). In patients without diabetes the correlation of serum HTG concentration with serum albumin level was $r = -0.330$ *(*$p < 0.030$*), with platelet count was* $r = 0.320$ *(*$p < 0.040$*), with serum iron concentration was* $r = -0.450$ *(*$p < 0.002$*), with TSAT was* $r = -0.4200$ *(*$p < 0.005$*), and with age was* $r = 0.337$ *(*$p = 0.003$*). No such relationships were seen in patients with diabetes. In both subgroups, no dependence was seen between serum HTG concentration and weekly Kt/V, total weekly CCr, or serum cholesterol concentration.*

Serum HTG concentration in PD patients without diabetes may be a valid inflammatory marker. The HTG serum level displays a significant statistical dependence on age, platelet count, and markers of nutrition such as serum albumin level, iron, and TSAT. It does not depend on markers of dialysis adequacy (weekly Kt/V, total weekly CCr) or on serum cholesterol concentration. The serum HTG concentration in PD patients with diabetes is lower than that in patients without diabetes, and it is not related to examined factors of inflammation, nutrition, or adequacy of dialysis.

Key words
Haptoglobin, diabetes, adequacy of dialysis, nutrition status

Introduction

End-stage renal disease (ESRD) is characterized by a high mortality rate (1), higher levels of pro-inflammatory cytokines, and increased oxidative stress (2), all of which also are connected with malnutrition and atherosclerosis (3). Inflammation predicts poor outcome in ESRD. The acute-phase response may also be related to oxidative stress and progressive vascular injury and consequently may contribute to cardiovascular disease (CVD).

Haptoglobin (HTG) is a hemoglobin (Hb)–binding protein. Hemoglobin is released during erythrocyte disintegration. The Hb-HTG complex is rapidly removed from the circulation. Intravascular hemolysis increases the release of Hb, which in turn reduces serum HTG concentration. During acute hemolysis, HTG is totally consumed.

As a Hb-binding protein, HTG provides protection against the oxidative stress (4) that plays an important role in the development of diabetic vascular complications (5). But haptoglobin is also an acute-phase protein that can rise in response to inflammation.

The aim of the present investigation was to find any relationship between serum HTG concentration,

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adequacy of peritoneal dialysis (PD), and the nutrition status of PD patients with and without diabetes.

Patients and methods

The 60 patients (36 men, 24 women) in our study had been on dialysis (54 on continuous ambulatory PD, 6 on automated PD) for a mean of 17 months (median: 12 months; range: 0.5 – 66 months). The age range of the patients was 18 – 77 years (mean: 53 years). We divided this group of patients into two subgroups: those with diabetes [$n = 17$; mean age: 54 ± 14 years; treated with PD through 2 – 66 months (median: 11 months)] and those without diabetes [$n = 43$; mean age: 55 ± 15 years; treated with PD through 0.5 – 65 months (median: 14 months)].

Serum concentration of HTG was determined by immunonephelometry (Behring Nephelometer Analyzer: Behringwerte AG, Marbus, Germany). The following parameters were simultaneously measured: serum albumin level, platelet count, serum iron concentration, transferrin saturation (TSAT), weekly Kt/V, total weekly creatinine clearance (CCr), and serum cholesterol concentration. Statistical analysis of the data [computation of the mean, standard deviation (SD), variance, kurtosis, and median; comparison of the observed distribution of variables against the normal distribution; statistical significance tests for evaluating the discrepancy of the observed data from the respective theoretical distributions] was performed using Statistica, version 6.0 (StatSoft, Tulsa, OK, U.S.A.).

Results

The reference range of serum HTG concentration is 0.3 – 2.0 g/L. This "normal range" was seen in only 25 patients. The mean HTG concentration for the entire group (2.4 ± 1.2 g/L) was above the norm. The distribution of serum HTG concentration was not normal in the group of patients under investigation (Figure 1 histogram; chi-square test: $\chi^2 = 14.14$; $df = 5$; kurtosis = 0.38). Figure 2, in which histograms for both subgroups are presented (that is, for patients with and without diabetes), clarifies that finding. The mean ± SD serum HTG concentration measured 2.0 ± 1.1 g/L (with diabetes) and 2.5 ± 1.2 g/L (without diabetes). Figure 3 shows the difference between the medians of the two subgroups. That difference is statistically significant. Table I gives the results of the four nonparametric tests and their significance levels.

Additionally, in the subgroup of patients without diabetes, significant statistical dependencies (Spearman test) were noted between HTG concentration and age, serum albumin level, platelet count, serum iron concentration, and TSAT (Figures 4 – 8). Table II shows the Spearman coefficients *(r)* and significance levels. Such dependences were not obtained in the subgroup of patients with diabetes.

Discussion

In ESRD patients, CVD mortality is 10 – 20 times higher than in the general population (6). Additionally, 30% – 50% of ESRD patients, including PD patients, have elevated serum levels of C-reactive protein

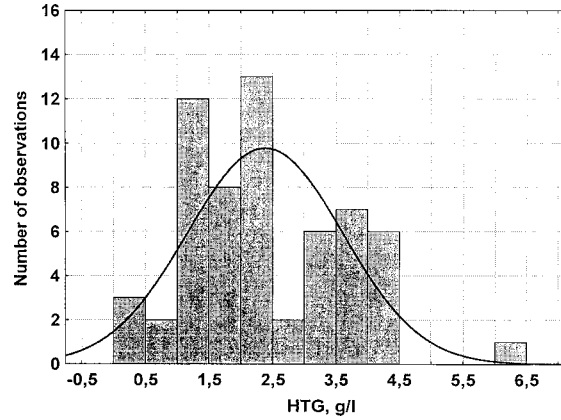

FIGURE 1 Histogram of the serum haptoglobin (HTG) concentration (g/L) in the entire group of examined patients.

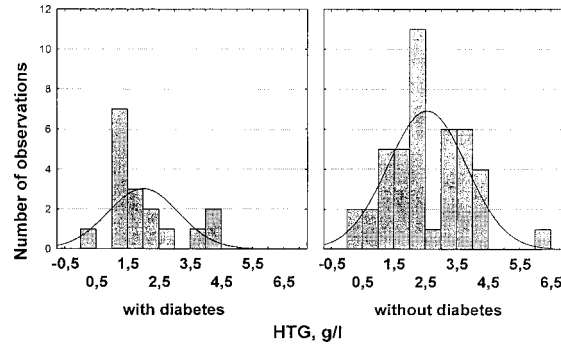

FIGURE 2 Histogram of the serum haptoglobin (HTG) concentration (g/L) in the subgroups of patients with and without diabetes.

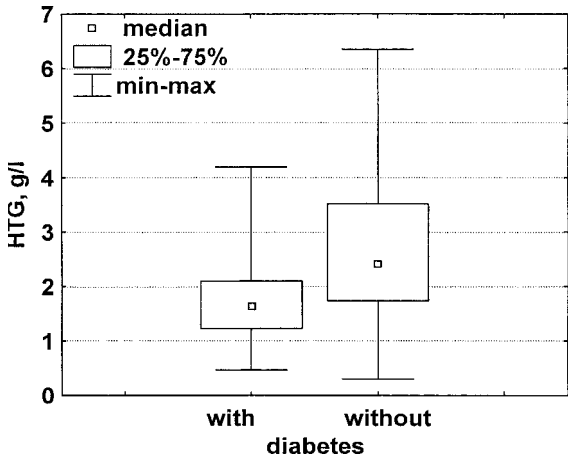

FIGURE 3 Median and range of the serum haptoglobin (HTG) concentration (g/L) in patients with and without diabetes.

TABLE I Results of nonparametric tests rejecting the hypothesis that no difference exists between mean serum haptoglobin concentrations in patients with and without diabetes on the given significance level *p*

Wald–Wolfowitz test	$p=0.040$
Kolmogorov-Smirnov test	$p<0.050$
Mann–Whitney *U*-test	$p=0.092$
Kruskal–Wallis test	$p<0.010$

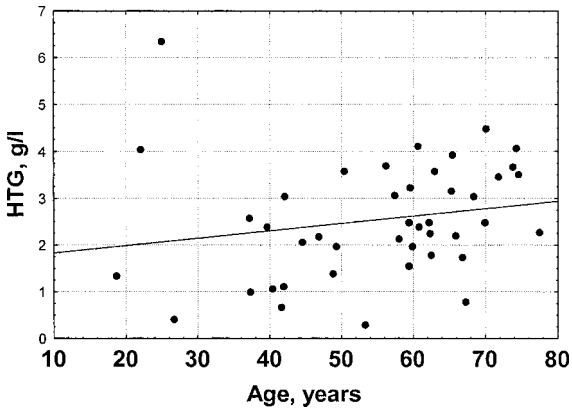

FIGURE 4 Serum haptoglobin (HTG) concentration (g/L) versus age (years) in patients without diabetes.

(7). A relationship between inflammation, oxidative stress, and higher frequency of CVD is suggested in ESRD patients (7,8).

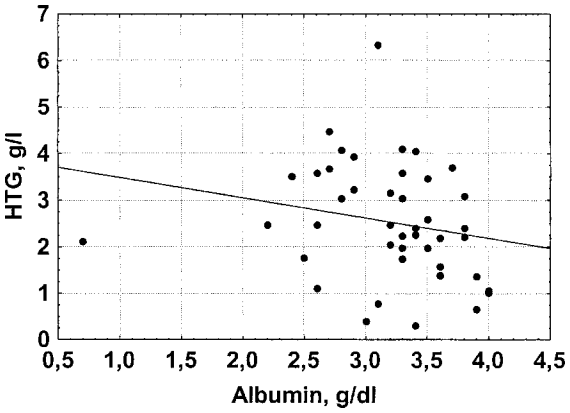

FIGURE 5 Serum haptoglobin (HTG) concentration (g/L) versus serum albumin concentration (g/dL) in patients without diabetes.

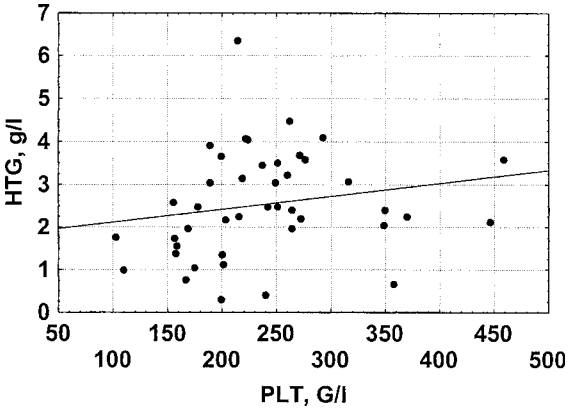

FIGURE 6 Serum haptoglobin (HTG) concentration (g/L) versus platelet (PLT) count (g/L) in patients without diabetes.

Some recent reports have pointed to the existence of relationships between inflammation-sensitive plasma proteins (ISPs: fibrinogen, α1-antitrypsin, HTG, ceruloplasmin, orosomucoid) and systolic blood pressure (SBP) or incidence of stroke (9). The dependencies were established in a large cohort of healthy men with a long follow-up. The authors concluded that high ISP levels are associated with elevated SBP and increased risk of stroke. The same authors noted that hypercholesterolemia, which increases the cholesterol-related incidence of CVD, is associated with high plasma levels of ISPs (10). Other authors have examined certain acute-phase proteins (amyloid A,

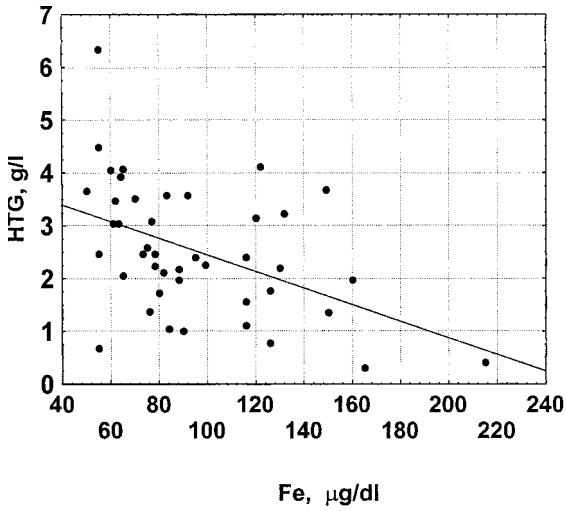

FIGURE 7 Serum haptoglobin (HTG) concentration (g/L) versus serum iron (Fe) concentration (μg/dL) in patients without diabetes.

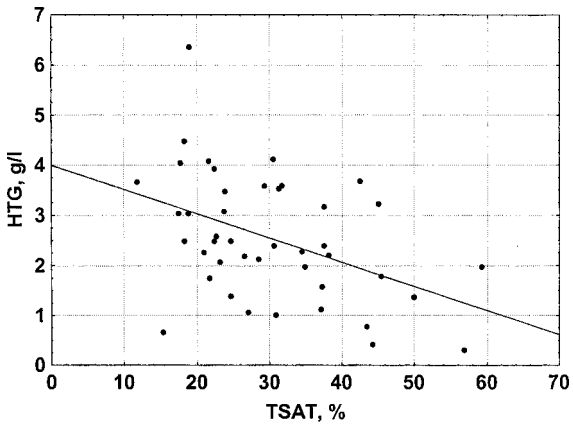

FIGURE 8 Serum haptoglobin (HTG) concentration (g/L) versus transferrin saturation [TSAT (%)] in patients without diabetes.

TABLE II Spearman coefficients *(r)* and significance levels *(p)* of haptoglobin relationships to age, serum concentration of albumin, platelet count, serum iron, and transferrin saturation

Age	$r=0.337$	$p<0.003$
Serum albumin concentration	$r=-0.330$	$p<0.030$
Platelet count	$r=0.320$	$p<0.040$
Serum iron	$r=-0.450$	$p<0.002$
Transferrin saturation	$r=-0.420$	$p<0.005$

serum C-reactive protein, plasma fibrinogen, and serum HTG) as markers for coronary heart disease (CHD) in a middle-aged male population. In their conclusion, those authors preferred serum C-reactive protein as a first-class acute-phase reactant for detection of CHD (11).

In serum, HTG is a marker of inflammation and an antioxidant factor (4). In the present study, significant differences were seen in the HTG serum level of patients with and without diabetes. Further study is needed to explain the reasons for that state of affairs. In patients with diabetes, an increase in oxidative stress seems to be responsible for the lower serum concentrations of HTG and its behavior (atypical for an inflammatory factor, not being correlated with other markers of inflammation and nutrition).

Conclusions

1. Serum HTG concentration in PD patients without diabetes is elevated and may be a typical inflammatory marker.
2. Serum HTG concentration in PD patients without diabetes displays a significant statistical dependence on age and other markers of inflammation and nutrition such as serum albumin level, platelet count, serum iron concentration, and TSAT.
3. Serum HTG concentration in PD patients with diabetes is lower than in patients without diabetes and is unrelated to the inflammation and nutrition factors mentioned in point 2.

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Systemic Inflammation Induces Endothelial Dysfunction in Peritoneal Dialysis Patients.

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Dr. López-Cabrera and Rafael Selgas collaborate equal to perform the present study.

Running Title: Inflammation and endothelial dysfunction.

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Background. Endothelial dysfunction (ED) with atherosclerosis is a recognized complication of uremic patients. The importance of inflammation in the ED pathophysiology has recently been proposed. The aim of this study was to analyze the role played by inflammation as starter of ED in peritoneal dialysis (PD) patients.

Methods. During 15 months, all the patients from our PD unit were followed-up. We determined Nutritional, inflammatory (C-reactive protein (CRP), TNF-α and Vascular cell adhesion molecule-1 (VCAM)) and endothelial function markers at baseline and during systemic inflammation (SI). Seventeen patients were finally included due to elevation of CRP by various etiologies (four suffered silent infection by *Helicobacter pylori*, four upper respiratory infections and two intestinal bacterial over-growth). They were compared with a control group (CG) with 12 PD patients who did not suffer SI.

A venous oclusion test (VOT) was performed to stimulate the endothelium by inducing a stasis in the right arm. Endothelial fibrinolytic capacity: tissue-type plasminogen activator (t-PA) and plaminogen activator inhibitor (PAI). Endothelial damage markers: von Willebrand factor (vWF), thrombomodulin (TM), and nitric oxide (NO). Cardiovascular (CV) risk markers: fibrinogen, Lipoprotein(a) and homocysteine (Hcy). Growth factors: VCAM-1, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF). After variable of follow-up time, C-RP and TNF-α plasma levels increased.

Results. We found a decrease in albumin, tPA ratio (post VOT/pre-VOT) and NO_3-ratio. PAI, TM, Lp(a) and TGF-β Increase. A positive linear correlation between Hcy and PDGF was found, suggesting a pro-atherogenic environment. CG did not suffer modifications in no studied parameters.

Conclusion: In PD patients, systemic inflammation induces endothelial dysfunction estimated by elevation of endothelial damage markers. Pro-inflammatory cytokines are deeply associated to elevation of procogulable and proatherosclerotic mediators in plasma.

Key Words: Systemic Inflammation. Endothelial Dysfunction. Cardiovascular Risk. Peritoneal Dialysis. MIA syndrome.

Introduction

Endothelial dysfunction (ED) is a common element in atherosclerosis and hypertension and is considered an independent cardiovascular (CV) risk factor (1). It is also a prominent feature of uremic status and protein malnutrition (2, 3). ED intrinsically may induce procoagulant events (4). Procoagulant status is recognized as a atherosclerosis promoter (1, 3-5).

On the basis that malnutrition and cardiovascular complications are the main cause of morbidity and mortality in dialysis patients (2), it has recently been suggested that malnutrition and accelerated atherosclerosis might be part of the same complex syndrome, the so-called MIA (malnutrition, inflammation and atherosclerosis) syndrome. MIA syndrome (5) adds to the traditional CV risk factors (hypertension, dyslipemia, smoking, obesity and sedentary), the inflammation as a central and crucial part (6). Although a lot of information exists as to the relationship between inflammation, ED, atherosclerosis and CV complications in renal (6) and non-renal patients (7), the exact point in the pathogenesis of MIA syndrome remains undefined. Several questions that need response include whether inflammation is the key and starting factor responsible for stimulating factors implicated in atherosclerosis cascade, whether inflammation is a cause or consequence and whether inflammation is a silent constant or cyclic process.

The aim of the present study was to define the role played by inflammation as starting factor for ED, subsequent malnutrition and CV risk, convincing to constitute a sole syndrome in peritoneal dialysis (PD) patients.

Patients and Methods

During 15 months, we followed-up all patients from our PD unit (n= 68, 17 initiating dialysis) to periodically determine inflammatory, endothelial function and nutritional markers. All the parameters considered in this study at baseline and when inflammatory markers showed elevation. Therefore, the sole inclusion criteria was the elevation of C-reactive protein (CRP) from its baseline level or enhanced over the normal range, with elevation of one proinflammatory cytokine, along the study. Every month we also measured inflammatory markers, and if they suffered elevation, we immediately included the patient and measured endothelial function markers. We excluded patients with recognized active systemic diseases (systemic lupus, vasculitis or other active immune disease), as well as those with immunosuppression. We also excluded patients who started r-HuEPO by its potential effect on endothelium (8). However, eleven patients using regularly r-HuEPO (range 9-34 months) were admitted.

Study design: Seventeen patients, who suffered elevation of CRP and TNF-α after variable periods of time (1-15 months), were finally selected. Twelve were males and ten women, with an age range of 25–78 years (53 ± 11.2). We performed a baseline determination and follow-up of the patients determining periodically every month the C-RP (figure 1). The timing of the second determinations was just after C-RP showed elevation.

In regard to study group, the mean period on PD was 28.7 ± 27 months (range 1-117). The causes of renal failure were nephrosclerosis in five, diabetes in five, glomerulonephritis in three, polycystic kidney disease in two, tubulointerstitial disease

in one and unknown origin in one case. Eleven patients had been diagnosed of hypertension. As medical treatment, five were receiving angiotensin-converting enzyme (ACE) inhibitors, two calcium channel blockers, and six other drugs or different combinations. By echocardiogram eleven patients showed mild left ventricular hyperthrophy (LVH) (Penn convention criteria), five moderate LVH and five severe; four patients did not show LVH. Five used aspirin and three pentoxifillin. Active smoking was present in four patients and past history of smoking in seven.

We defined systemic inflammation (SI) as the elevation of CRP and one of the high sensibility inflammatory markers, TNF-α and vascular cell adhesion molecule-1 or VCAM-1 (9, 10).

We studied the possible cause for systemic inflammation (SI), including anamnesis, laboratory and image-diagnosis tests addressed by clinical data. As a consequence, we have diagnosed in four patients silent infection by *Helicobacter pylori* (breath test), four patients upper viral respiratory infections, and two intestinal bacterial over-growth (breath test). We were not able to establish the cause for increment of inflammatory markers in the remaining patients. The different studied parameters before and after suffering SI were contrasted, being the patients the control of themselves.

We also included a **control group** with 12 patients who did not suffer elevation of inflammatory markers. The different studied parameters were determined at baseline and between 1 and 3 months later (figure 1).

We determined the follow parameters:

Dialysis adequacy was assessed by weekly urea Kt/V and nPNA.

Peritoneal membrane transport. Urea and creatinine mass transfer coefficients (urea-MTC and Cr-MTC) were measured using standard method (11). Ultrafiltration (UF) capacity was calculated by a peritoneal exchange of 4 h using 3.86% glucose.

Nutritional markers included serum albumin (colorimetric method), cholesterol (Hitachi 704), transferrin and prealbumin by immunonephelometric methods (Boehring, Nephelometer terminal SA.: Behringwerte AG, Magnus Germany).

Inflammatory markers were C-reactive protein (CRP) by immunonephelometric method (ELISA, Vectastin: vector laboratories, Burlingame, CA, USA.), tumor necrosis factor alpha (TNF-α) (Easia: Medgenix Diagnostics SA., Fleurus, Belgium) and VCAM-1 (Human Quantikine, R & D systems, MN, USA).

Venous oclusion test (VOT) was performed to stimulate endothelium, by inducing a stasis in the rightarm during 10 minutes applying a sphygmomanometer cuff inflated to a pressure midway between systolic and diastolic values [mean arterial pressure = (systolic pressure + diastolic pressure)/2] (8).

Endothelial factors associated with endothelial fibrinolytic capacity included tissue-type plasminogen activator (t-PA) pre-VOT and post-VOT, (ELISA-plasma-t-PA antigenic coaliza t-PA: chromogenix, Mölndal, Sweden), and PAI (plasminogen activator inhibitor) levels determined by a commercial monoclonal ELISA (Tintelize, PAI Biopool, Umea, Sweden). We also calculated t-PA ratio (t-PA post-VOT/pre-VOT).

Endothelial damage markers were von Willebrand factor (vWF) (asserachrom vWF: Boehringer Mannheim, Meylan, France), Trombomodulin (Asserachrom TM: Diagnostica Stago, Asnières, France), endothelial relaxing factor nitric oxide (NO) measured as nitrate (NO_3) concentration pre-VOT and post VOT by capillary electrophoresis. We also calculated NO_3-ratio: (post-VOT/pre-VOT).

Growth factors included vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF), all determined by ELISA commercial kits (Human Quantikine, R & D systems, MN, USA).

Other cardiovascular risk markers included serum or plasma levels of fibrinogen (inflammatory and pro-coagulant marker) (thrombin time method described by Clauss), lipoprotein-a (Lp(a)) (sandwich-type enzyme-linked immunoassay, TintElize Lp(a); Biopool, Umea Swede), and homocysteine (Hcy) (high pressure liquid chromatography). Blood samples were drawn in resting and fasting conditions, between 09:00h and 11:00h and collected into vacutainer tubes (Becton Dickinson) containing 0.129 mmol of sodium citrate. The samples were centrifuged and tested immediately or stored at –70ºC until assayed.

Statistic analysis. Results are given as mean (± SD) and range. Comparisons between groups were performed using "t" student tests for paired and non-paired data, and simple liner regression analysis was performed to investigate the relationship between all endothelial function and inflammatory markers. A "p" value less than 0.05 was considered statistically significant.

Results

The 17 selected patients showed Hb 11 ± 0.56 g/dL, systolic blood pressure (SBP) 130 ± 18 mmHg, diastolic blood pressure (DBP) 80 ± 11.1 mmHg, cholesterol 200.1 ± 38 mg/dL, transferrin 220 ± 36 mg/dL. Table I, shows the baseline data and the changes suffered by patients prior and during SI. A relatively elevated plasma levels of inflammatory markers (CRP and TNF-α) were found, C-RP 1.19 ± 2.38 mg/dL (range 0.3-5.2), (normal range 0.1-0.6), and TNF-α 38 ± 12.9 pg/ml (normal 2-20). These markers showed an important elevation after variable time, CRP enhanced to 3.8 ± 2.1, $p<0.05$, and TNF-α to 68.3 ± 16, $p<0.01$. 17 from the 22 studied patients showed CRP values into the normal range (0.32 ± 0.18 mg/dL), but only two patients showed normal

levels of TNF-α at baseline (range 12-68 pg/mL).Table II, shows the baseline and the changes in the same analysed parameters.

In regard to nutritional markers (albumin, prealbumin) and fibronolytic capacity markers (tPA-ratio, high PAI), they showed values lower than normal. In the right column, the values of these parameters after detecting the elevation of inflammation markers are shown. Inflammation was accompanied by a decrease in protein intake (nPNA), serum albumin and t-PA ratio (Table I).

The same schedule is used in Table II which shows the changes in endothelial function and damage markers. The increase in NO_3-pre VOT, trombomodulin, TGF-β and Lp(a) after the appearance of inflammation markers, is remarkable. Other factors showed elevation in the limit of the statistical significance.

To establish a relationship among these parameters, we performed a matrix of regression analysis both at baseline and after systemic inflammation (Table III). The baseline relationship between PDGF with Hcy and TGF-β with Lp(a) showed a narrowing ($r=0.4$, $p<0.05$), (0.6, $p<0.05$). We also found a negative statistically significant linear correlation between baseline TNF-α and NO_3-ratio (-0.3, $p<0.05$, after SI : -0.5, $p<0.05$), baseline TNF-α and tPA (-0.5, $p<0.05$), and positive between baseline TNF-α and VCAM-1 (0.5, $p<0.05$).

In relation to residual renal function (RRF), the mean CCr was 2.2 ± 2.1 ml/min at baseline. From 17 studied patients, 12 were anuric and eight showed RRF (CCr= 3.4 ± 2.1 ml/min). Anuric patients showed some baseline differences in analyzed parameters:

TNF-α and CCr showed a negative statistically significant linear correlation (-0.4, $p<0.05$). RRF seemed to represent a special protection in these patients with RRF.

Although, they showed similar changes in C-RP (1.1 ± 2 vs. 2.2 ± 1.27 mg/dL, $p<0.05$), TNF-α (31 ± 11 vs. 38.8 ± 7.8 pg/ml, $p<0.05$), NO_3-ratio (1.18 ± 0.21 vs. 1.48 ± 0.25, $p<0.05$), tPA (1.9 ± 1.4 vs. 1.55 ± 1.5, $p<0.05$) than that showed by anuric patients. TM showed no significant changes (263 ± 42 vs. 276 ± 38 ng/mL, NS). Nutritional markers, represented by serum albumin was higher in patients with RRF than anurics (3.8 ± 0.38 vs. 3.6 ± 0.4 mg/dL, $p<0.05$).

Finally, table IV shows the different markers measured in the control group. We did not find differences in all measured markers.

Discussion

The most important finding of this study is the establishment of a clear and real link between cyclic or constant episodes of SI with endothelial dysfunction, malnutrition and CV risk in PD patients.

Defining systemic inflammation (SI) in renal patients is very difficult because there is not a gold standard marker. One may suggest the CRP, the most used in clinical trials, but we consider this marker as a molecule appearing in late periods. IL-6 and TNF-α are retained by the lack of renal function in dialysis patients. For instance, in our series, 17 from 22 patients showed normal levels of CRP and only two showed normal TNF-α plasma levels. Are these patients suffering SI?. High levels of TNF-α may be the expression of only a mild grade of renal retention and it is difficult to give it a representative value of inflammation. We do not know which are the toxic levels of TNF-α in renal patients?. In a previous study we suggested that a value of TNF-α lower than 65 pg/ml defined the limit of tolerance, based in clinical manifestations (12). Therefore, any definition of SI in dialysis patients may be discussed. Finally, we have adopted the coincidental elevation of CRP and a pro-inflammatory cytokine plasma

level as definition of SI. This methodology has been used by others (9, 10), who used the elevation of CRP, IL-6 and MMP-9.

In fact, factors such as the contact of leucocytes with hemodialysis membrane are able to stimulate the cytokine production with deleterious effects (13). However, in many cases the elevation of these cytokines are not associated to symptoms and their tolerance level is not well known (12). CRP is the inflammatory marker mostly used in clinical trials, but this is at the end of inflammatory molecule cascade (13) and many early inflammatory presses may by infra-diagnosed.

On the other hand, we have confirmed the baseline existence of endothelial dysfunction in PD patients, characterized by a low fibrinolytic capacity, disorders in endothelial vasoactive function and elevated plasma markers of endothelial injury. All these features were associated with SI, ED and poor nutritional status (Table I-II, IV). These findings confirm the results of other investigators (5-7).

Low **fibrinolytic capacity** is characterized by tissue-type plasminogen activator (t-PA) deficiency. This is an endothelium-derived fibrinolytic glycoprotein released by exercise, VOT and stimulation with desmopresin. tPA values after stimulation by VOT (expressed as tPA-ratio), indicate an adequate fibrinolytic capacity (3, 8). It has been demonstrated that patients suffering severe atherosclerosis or unstable angina show low tPA levels after stimulation (14). Uremia *per se* is associated with poor tPA response for unknown causes (3, 4, 8). Recently, we have found that r-HuEPO treatment in PD patients is associated with a dramatic decrease in tPA-ratio post-VOT, causing a theoretical worsening of the uremia prothrombotic status (8). Importantly, we have found a clear association between elevated pro-inflammatory molecules (TNF-α) and a decrease in tPA-ratio (table I).

Experimentally, the stimulation of human endothelial cells from umbilical vein endothelial culture with IL-1 and TNF-α, reduces tPA synthesis (15). Since tPA can also be considered as a pro-inflammatory molecule (15, 16), the spontaneous or post-VOT elevation of tPA may result in a decrease of tPA-ratio and an increase in PAI levels, predisposing to coagulant phenomena (3, 4, 8). Effectively, we have found that patients who suffered SI showed an important decrease of tPA-ratio, although, both tPA-post and pre VOT showed an increase (Table I). Chia S et al (16), found an increase in spontaneous tPA after SI and concluded, that SI invokes a protective response mediated by enhancing endothelial fibrinolytic capacity. However, they did not measure tPA-post endothelial stimuli (i,e. VOT) which could be a better method to measure tPA-activity (8).

Another important fibrinolytic inhibitor, the PAI, resulted augmented by inflammation. It has been demonstrated *in vivo* and *in vitro* that cytokines (IL-1 and TNF-α) induce elevation of PAI (4, 6, 17). Moreover, malnourishment and patients with inflammation, show hepatic acute hyperproduction of half-life proteins such as C-reactant protein, prealbumin, fibronectin, PAI and others (5-7). Finally, and according to our results and those shown by longer series (18), the elevation of PAI associated with inflammation should be considered as a CV risk factor due to the predisposition to thrombotic events.

Endothelial damage as consequence of injuries is followed by vWF, TM and NO release (9, 18, 19). The vWF release causes stimulation of platelet aggregation and thrombus formation (2, 8, 17). In dialysis patients, elevated vWF plasma levels have been found spontaneously (8, 17). However, the kidneys do not remove vWF, and the lack of renal excretion should not affect plasma levels (19). vWF is also an acute phase reactant whose synthesis is increased by cytokines (20). In our study, no changes were

found in vWF levels after SI (table II), suggesting that other uremia-related factors (thrombin levels, fibrin monomers, endotoxins, complement, or some renal disease as glomerulonephritis), regulate vWF plasma concentration (19). TM is a structural protein from the endothelial cells membrane, released in injury conditions, which represents the dead cells burden (17). Due to its renal excretion, high plasma levels are found in uremia (8, 19). TM released in plasma activates protein-C to inhibit fibrin formation (17). TM plasma levels are also affected by inflammatory molecules, proteolysis and oxidative stress. In culture cells, the stimulation with high doses of TNF-α induces a dramatic increase in TM (4). Our results confirm that SI induces an increase in TM, representing endothelial damage. NO is the most potent vasodilator synthesized by endothelial cells, able to inhibit platelet adhesion, releases mitogenic factors, and causes proliferation of muscle vessel cells (21). Deficient NO production and early atherosclerosis (22) have been found after administration of recombinant TNF-α (23). Moreover, in endothelial cell culture, TNF-α administration reduces the half-life of mRNA encoding for NO-synthase (24), predisposing to NO-depend vasoconstriction (25). Effectively, after suffering SI, patients showed spontaneous elevation of NO_3-pre VOT with a low response capacity demonstrated by NO_3-post VOT, and in consequence a decrease in NO_3-ratio (VOT, NO_3-post/-pre) (Table II).

Endothelial remodeling, there is growing evidence about the implication of growth factors in endothelial dysfunction and atherosclerosis pathway. These show an important proliferating activity and are responsible of collateral circulation development (26). TGF-β is a double edge sword growth factor because in normal conditions it operates as anti-inflammatory, but in pathologic situations it acts as a profibrotic agent. In advanced atherosclerosis TGF-β plasma levels are characteristically diminished (26). Consistent with low growth factor plasma levels found in atherosclerosis is the concept

that atherosclerosis is a low-remodeling status. We have found an increase of TGF-β plasma levels (table II) after SI and a positive correlation between TGF-β and PAI (Table III). TGF-β stimulates PAI production especially under inflammation situations (27, 28) and decreases fibrinolytic capacity (26). One of the mechanisms by which TGF-β induces endothelial damage is by increasing PAI. The elevation of TGF-β could represent an acute anti-inflammatory counter balance. Moreover the inflammatory response syndrome induces synthesis and releases pro and anti-inflammatory cytokines including TGF-β (28).

Lipoprotein$_{(a)}$ and homocysteine are recognized **CV risk factors** (29) frequently elevated in dialysis patients (4, 7, 29). Inflammation causes an increase in the hepatic synthesis of acute phase reactants. Lp(a) is one of these proteins able to induce procoagulable status (30). Halper et al (31), in non-uremic population described that the negative association of TGF-β and Lp(a) is very proatherogenic. We found a spontaneous tendency to a positive linear correlation between both molecules in our series. Moreover, after inflammation this relationship increased in significance (table III), suggesting that acute TGF-β rise differentiates from what happens in chronic processes such as atherosclerosis where TGF-β plasma levels decreased and Lp(a) maintained elevated levels although patients showed inflammatory signs (32). In addition, it has been demonstrated that persistent high Lp(a) level is able to inhibit TGF-β generation in smooth muscle cells (31, 32).

Homocysteine may also be affected by inflammation (4, 17, 29). However, we did not find differences in Hcy plasma levels after SI (table II). Hcy is able to induce ED and atherosclerosis by several mechanisms, stimulating the vessel smooth cell proliferation and mitogenesis, and platelet adhesion and through its relationship with

PDGF (33). Our results showed a significant linear correlation between Hcy and PDGF ($r= 0.56$, $p<0.05$) (table III) after suffering SI, supporting this mechanism.

Importantly, we did not find any change in the control group (table IV), indicating the inflammation is the specific factor responsible of triggering cachexia and pro-atherogenic mechanisms in uremia.

The role of **RRF** in the elimination of the excess of pro-inflammatory molecules has not provoked much attention (34). Moreover, the current dialysis methods do not eliminate adequately the cytokine overproduction. On the contrary, peritoneal membrane or hemodialysis filters are important sources of cytokine production (13). Other important sources are silent infections, virus, *Chlamydia pneumonie, Helicobacter pylori,* dental infections, vascular access with thrombosis and native kidneys (13, 35). Our findings support the idea that RRF protects dialysis patients from the excess of pro-inflammatory molecules, at least partially. Effectively, in our patients without RRF, the changes in endothelial damage and function markers caused by SI were more severe than in those with RRF.

In conclusion, systemic inflammation induces endothelial dysfunction increasing procogulable and proatherosclerotic mediators and endothelial damage markers in peritoneal dialysis patients. The present research confirms the link between the different parts of MIA syndrome and remarks the importance of systemic inflammation as a starting process of endothelial dysfunction, malnutrition and cardiovascular risk in uremia.

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Table I. Changes in Nutritional and Endothelial Function Markers During SI.

Parameter	Normal range	Prior SI	During SI	P
CCr (ml/min)	90-110	2.18 ± 2	1.9 ± 2.4	NS
Weekly-Kt/V-urea	>2	2.2 ± 0.38	2.22 ± 0.4	NS
nPNA (g/kg)	>1.1	1.25 ± 0.29	1.09 ± 0.34	<0.05
Albumin (mg/dL)	>4	3.89 ± 0.4	3.66 ± 0.44	<0.05
Prealbumin (mg/dL)	>30	32.8 ± 10	32 ± 12.5	NS
Urea-MTC (ml/min)	variable	17 ± 3.1	21.1 ± 5.8	NS
Cr-MTC (ml/min)	7-11	8.8 ± 1.9	10.6 ± 2.9	0.07(NS)
UF 3.86% (ml)*	variable	740 ± 253.3	766.4 ± 227	NS
PAI (ng/mL)	<10	9 ± 4.01	11.5 ± 3.5	NS
tPA-pre VOT (ng/mL)	1-12	8.8 ± 6.1	12.4 ± 5.1	0.05
tPA-post VOT (ng/mL)	variable	17.9 ± 7.2	16 ± 2.5	NS
tPA-ratio	>2	2.03 ± 1.18	1.29 ± 0.5	<0.05

CCr: creatinine clearance. C-RP: C-reactive protein. nPNA: normalized protein nitrogen appearance. *UF: peritoneal ultrafiltration with a 4h 3.86% dextrose. PAI: plasminogen activation inhibitor. t-PA: tissue-type plasminogen activator.

Table II. Changes in Endothelial Damage, Cardiovascular Risk Markers and Growth Factors During SI.

Parameter	Normal range	Prior SI	During SI	P
NO_3-pre VOT (μmol/L)	40-60	32.1 ± 16	52.8 ± 12.4	<0.05
NO_3-post VOT (μmol/L)	variable	52 ± 33	56 ± 37.1	NS
NO_3-ratio	variable	1.6 ± 2	1.06 ± 3	<0.05
vWF-pre VOT (%)	60-150	223 ± 41	227 ± 72	NS
TM (ng/mL)	14-55	279 ± 63	289 ± 47	<0.08(NS)
VCAM-1 (ng/mL)	395-714	1297 ± 409	1548 ± 198	<0.05
VEGF (pg/mL)	62-707	317 ± 153	400.3 ± 156	NS
TGF-β (pg/mL)	34-64	25.4 ± 8.4	34.7 ± 10.8	<0.05
PDGF (pg/mL)	750-1100	1500 ± 655	1617 ± 577	NS
Lp(a) (ng/mL)	<20	38.5 ± 9.9	56 ± 14.1	<0.05
Fbrinogen (mg/dL)	150-350	463 ± 111	485 ± 100.7	NS
Hcy (ng/mL)	5-10	32.3 ± 11.5	36.3 ± 18.1	NS

NO_3: nitrate. vWF: von Willebrand factor. TM: thrombomodulin. VCAM-1:Vascular cell adhesion molecule-1. VEGF: vascular endothelial growth factor. TGF-β: transforming growth factor betta. PDGF: platelet-derived growth factor. Lp(a): liprotein (a). Hcy: homocysteine

Table III. Matrix of Linear Correlations at Prior and During SI

Marker	tPA-ratio		Lp(a)		Hcy		NO_3-ratio		CRP	Fibri-nogen	Albu-min	PAI	VCAM-1
	Pre-SI	After SI	Pre-SI	After SI	Pre-SI	After SI	Pre-SI	After SI	Pre-SI	Pre-SI	Pre-SI	Pre-SI	Pre-SI
TNF-α	-0.5 *	-0.57 *			0.3 **	0.34 **	-0.3 *	-0.5 *	0.5 *	0.36 **	-0.48 *		0.5 *
PDGF					0.4 **	0.6 *				0.22 **			
TGF-β			0.34 **	0.5 *					0.36 *			0.66 *	

*: p<0.05. **: NS but near to statistical significance.

Table IV. Nutritional, Endothelial Function, cardiovascular and growth Factors in the Control Group.

Parameter	Baseline	1-3 months	P
CCr (ml/min)	3 ± 2.7	2.3 ± 3.1	NS
Weekly-Kt/V-urea	2.31 ± 0.31	2.3 ± 0.39	NS
nPNA (g/kg)	1.22 ± 0.28	1.23 ± 0.24	NS
Albumin (mg/dL)	3.77 ± 0.3	3.81 ± 0.32	NS
Prealbumin (mg/dL)	33 ± 8	31 ± 11.5	NS
Urea-MTC (ml/min)	18.2 ± 4.1	19.1 ± 6.1	NS
Cr-MTC (ml/min)	8 ± 2.1	9.1 ± 3.2	NS
C-reactive protein (mg/dL)	0.33 ± 0.1	0.25 ± 0.2	NS
TNF-α (pg/mL)	31.8 ± 12	28.1 ± 14	NS
PAI (ng/mL)	8.1 ± 3	7.8 ± 4.5	NS
tPA-ratio	1.88 ± 3	2.2 ± 1.1	NS
NO_3-ratio	1 ± 0.8	1.7 ± 1.2	0.07 (NS)
vWF-pre VOT (%)	331 ± 123	278 ± 156	NS
TM (ng/mL)	301 ± 112	331 ± 79	NS
VCAM-1 (ng/mL)	1341 ± 487	1409 ± 501	NS
VEGF (pg/mL)	505 ± 398	663 ± 407	NS
TGF-β (pg/mL)	38.2 ± 12.8	42 ± 15.1	NS
PDGF (pg/mL)	999 ± 591	1204 ± 661	NS
Lp(a) (ng/mL)	41 ± 12.1	33.5 ± 9	0.09 (NS)
Fbrinogen (mg/dL)	413 ± 89	366 ± 97	NS
Hcy (ng/mL)	28.1 ± 9.1	34 ± 12.1	NS

CCr: creatinine clearance. TNF-α: tumor necrosis factor alpha. PAI: plasminogen activation inhibitor. t-PA: tissue-type plasminogen activator. NO_3: nitrate. vWF: von Willebrand factor. TM: thrombomodulin. VCAM-1:Vascular cell adhesion molecule-1. VEGF: vascular endothelial growth factor. TGF-β: transforming growth factor betta. PDGF: platelet-derived growth factor. Lp(a): liprotein (a). Hcy: homocysteine

Figure legend.

Figure 1. Study design, we study 68 clinically stable peritoneal dialysis patients. We performed baseline endothelial function test in all of them. 46 patients were excluded of the follow-up because these did not show elevation of C-reactive protein (CRP) or pro-inflammatory cytokines. 22 patients showed elevation of inflammatory markers. Five of 22 them initiated the study with CRP mildly elevated therefore were excluded. Finally, 17 patients were included and follow-up during 15 months. When we detected elevation of CRP we repeated endothelial function test. Control group 12 patients whom did not suffered elevation of inflammatory markers. We determined inflammatory, endothelial function, growth factors and nutritional markers baseline and between 1 and 3 months later.

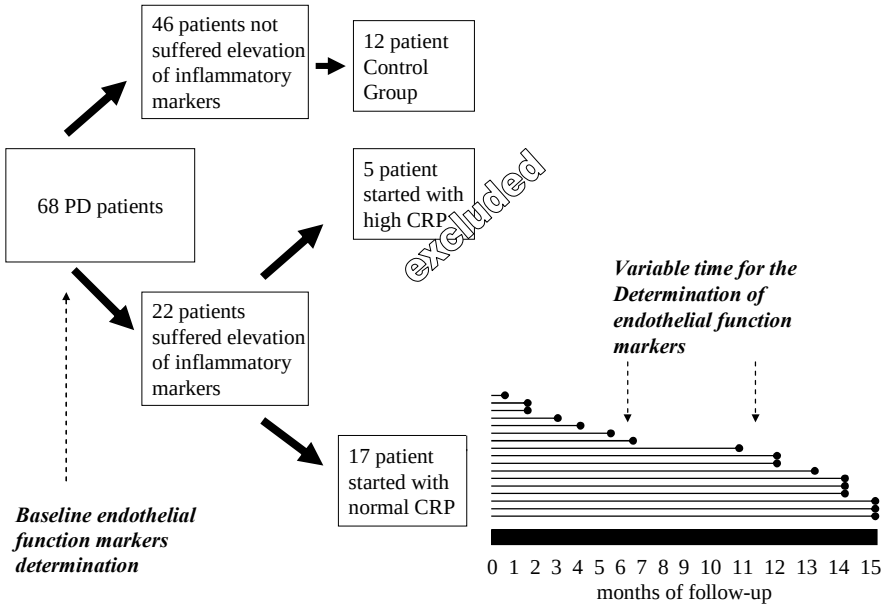
46 patients not suffered elevation of inflammatory markers
12 patient Control Group
68 PD patients
5 patient started with high CRP
excluded
Variable time for the Determination of endothelial function markers
22 patients suffered elevation of inflammatory markers
17 patient started with normal CRP
Baseline endothelial function markers determination
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
months of follow-up

Fig. 1.

Helicobacter pylori Eradication Improves the Malnutrition, Inflammation and Atherosclerosis Syndrome in Peritoneal Dialysis Patients

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FINAL

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Abstract: Background. *Helicobacter pylori* (*Hp*) infection has frequently been detected in dialysis patients. Chronic infection induces cytokine production which is poorly cleared by inefficient kidneys. These cytokines have systemic and catabolic effects. We studied the effects of *Hp* infection-eradication on serum cytokine levels, nutrition and endothelial function (EF) markers in peritoneal dialysis (PD) patients.

Methods. *Hp*-infection was diagnosed by breath test. Prior and post eradication we measured, nutritional markers: biochemical, daily food-intake and appetite modulator (orexigen neuropeptide-Y, NPY, anorexigen cholecystokinin, CCK). Stomach acid secretion: pepsinogen-I and II, and inflammatory markers: C-reactive protein (CRP), plasma TNF-α and IL-6. And EF markers which were taken pre- and post-venous occlusion test (VOT) in the right-arm for 10 min: tissue-type plasminogen activator (tPA), NO_3 (representing nitric oxide) and plasminogen activator inhibitor (PAI).

Forty-eight clinically stable PD patients divided into four groups according to *Hp*- infection and food intake were studied: I, *Hp*(+) and low food-intake (<30 kcal/kg/day, DOQI-guidelines), n=12; II, *Hp*(+) and normal food intake, n=4, III *Hp*(-) and low food intake, n=5, IV, *Hp*(-) and normal food intake, n=27.

Group-I showed the highest cytokines and the lowest residual renal function (RRF). TNF-α: group-I 127±85.5* pg/ml; group-II 70.5±25; group-III 60.5±10* and group-IV 43.4±5.4*, *p<0.05. IL-6: group-I 34.2±18* pg/ml, group-II 3.4±7*, *p<0.05, group-III 11.1±7.9*, *p<0.05 and group-IV 1.02±0.65*, *p<0.05. RRF was significantly higher in group-IV (4.8±1.6 ml/min*) than group-I (2.7±2.3*, *p<0.05). After *Hp*-eradication in group-I, nutritional markers and NPY increased. Inflammatory, gastric acid-secretion parameters decreased. EF markers also improved. Control group included 10 volunteers, non-renal subjects infected by *Hp* who followed similar process, including eradication treatment. *Hp*-eradication was associated to improvement in inflammatory and stomach acid-secretion markers.

Conclusions. *Hp*-infection induces cytokine overproduction associated with malnutrition, inflammation and atherosclerosis syndrome in PD patients. *Hp*-eradication normalizes stomach acid secretion, decreases inflammation and improves nutritional status and EF.

Keywords: Peritoneal dialysis, MIA syndrome, *Helicobacter pylori*, systemic inflammation, cytokines.

INTRODUCTION

The role of inflammation in the genesis of uremic cachexia and atherosclerosis has recently been emphasized [1]. This hypothesis, so-called as Malnutrition, inflammation and atherosclerosis syndrome (MIA) include a complex syndrome that meets malnutrition, high circulating levels of inflammatory molecules and atherosclerosis, and divide the patients in two specific groups, MIA-type I, with normal or low albumin, co-morbidity uncommon, absence of inflammatory markers, low food intake, normal resting energy expenditure (REE), increased oxidative stress, decrease in protein catabolism, which is possibly reversible with dialysis and nutritional support.

MIA-type II shows low albumin, high co-morbidity, high inflammation markers, low or normal food intake, elevated REE, markedly elevated oxidative stress, increase in protein catabolism and non-reversible with dialysis or nutritional support. In the latter case, inflammation, alone or in combination with low protein intake, would play the major role.

MIA syndrome [2] adds to the traditional Cardiovascular (CV) risk factors (hypertension, dyslipidemia, smoking, obesity and sedentary), the inflammation as a central and crucial part [3]. Although considerable information exists as to the relationship between inflammation, endothelial dysfunction, atherosclerosis and CV complications in renal [3] and non-renal patients [4], the essential or triggering factor in

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the pathogenesis of MIA syndrome remains undefined. Several questions that need an answer include whether inflammation is the key and initiating factor responsible for stimulating factors implicated in the atherosclerosis cascade, whether inflammation is a cause or consequence and whether inflammation is a silent, constant or intermittent pathologic process.

Hp is a chronic, often silent infection that induces chronic overproduction of IL-1, IL-6 and TNF-α in the non-uremic population [5-7]. Recently, it has been suggested an association between *Hp*-chronic infection, systemic and vascular inflammation, endothelial dysfunction [8], hypertension [9] and an increase in arterial stiffness (atherosclerosis) in a healthy population [8, 10].

In end stage renal disease, renal retention together with cytokine overproduction may be responsible for high plasma levels of cytokines [11-13]. Concurring with this hypothesis, the best model of renal-derived cachexia would be a silent infectious process operating in anuric patients.

The hypothesis of this study was that chronic silent *Hp* infection causes overproduction of pro-inflammatory cytokines which is exacerbated by the lack of renal elimination in uremic subjects. These extended periods of inflammation have cachectic and endothelial effects and lead to MIA syndrome in dialysis patients.

PATIENTS AND METHODS

This is a cross-sectional study including 48 non-selected peritoneal dialysis (PD) patients, 24 on continuous ambulatory peritoneal dialysis (CAPD) and 24 on automatic peritoneal dialysis (APD), 22 male and 26 female, ranging in age from 22 to 79 years (mean 56.9 ± 14). Patients with recognized endothelial diseases (vasculitis, scleroderma, malignant hypertension), active systemic disease and those under immunosuppression were excluded. The causes of renal failure were glomerulonephritis in nine cases, diabetes in eight, chronic pyelonephritis in ten, polycystic kidney disease in six, nephrosclerosis in ten, unknown etiology in four, and others in one. No recognizable acute or chronic active disorders were present in the three months prior to the study. The mean period on PD was 17.5 ± 13.2 months (range 3-63).

Hp infection was diagnosed by breath test (Taukit test; Isomed, Madrid, Spain). This method is based on oral intake of 100 mg of ^{13}C urea. In the presence of *Hp*, urease hydrolyzes ^{13}C urea, releasing $^{13}CO_2$, which is detected by mass spirometry in a breath sample. Patients were fasting and then drank half a glass of citric solution (200 cc). After 10 min, the baseline breath sample (exhalation) was obtained. A solution of Taukit plus water was ingested immediately and the second breath sample obtained 309 min later. The breath test for *Hp* infection diagnosis is a highly sensitive and specific method, currently the diagnostic method of choice [7].

Food intake was estimated using a three-day diet assessment (carbohydrates, lipid and proteins per day), including a weekend day (Wander; Sandoz Nutrición, 1990, Barcelona, Spain). Results are expressed in kcal/kg/day. According to DOQI guidelines for dialysis patients [14], a lower food intake is considered when daily food take is less than 30 kcal/kg/day.

Four groups of patients were constituted according to daily food intake and the presence or absence of *Hp* infection; group I, *Hp* positive with low daily food intake, (n = 12). Group II, *Hp* positive with normal food intake (n = 4). Group III, *Hp* negative with lower food intake (n = 5) and group IV, *Hp* negative with normal food intake (n=27).

Other GI symptoms included pyrosis (gastrointestinal reflux) and dyspepsia in 6 and 8 patients, respectively. Four patients from group I showed pyrosis and five dyspepsia. In the group II, two patients suffered dyspepsia. In the group III, two patient suffered pyrosis, and finally, two patients from group IV showed dyspepsia.

Groups I and II were treated to eradicate *Hp*. Two breath tests, at baseline and after treatment, were used for confirmation. Cytokine levels from patients in whom treatment was ineffective were not included in this part of the study. The therapeutic regime included omeprozole (40 mg/day), clarithromycin (1 g/day) and amoxocillin (1 g/day) with clavulanic acid (250 mg/day) for 10 days.

Gastro duodenal endoscopy addressed by GI symptoms was performed in ten patients. None of patients included in this study showed gastric or duodenal lesions (ulcer or gastritis).

A control group was included (10 volunteers, non-renal population) infected by *Hp* who followed similar process, including eradication treatment.

Dialysis adequacy was assessed by weekly urea-KT/V (urea-kinetic) and nPNA (normalized nitrogen-protein equivalence appearing) [15]. Creatinine renal clearance (Ccr) was also calculated.

Nutritional markers were determined by measuring serum albumin by the colorimetric method (Hitachi 704), transferrin, and prealbumin by the immunonephelometric method (Boehring, Nephelometer-Terminal, Spain. Normal range was 10-40 mg/dl). IGF-I was measured by radioimmunoassay (RIA) (Nichols Institute Diagnostics, San Juan Capistrano, CA), with a normal range of 83-450 ng/ml for persons <40 years and from 54-389 ng/ml for >40 years. Cholesterol and triglycerides were measured by a colorimetric method and Hitachi 704, respectively. The normal range for cholesterol was 150-250 mg/dl and triglycerides, 50-175 mg/dl. Ferritin and iron were also determined using a Hitachi 911.

Appetite Modulators

Neuropeptide Y (NPY) is the most potent orexigen known. NPY was measured in fasting conditions by RIA method (Peninsula Lab., Belmont, CA) sensitivity of IC_{50}: 23 pg/tube; specificity for human, porcine and rat NPY was 100%. No cross-reactivity was detected for pancreatic polypeptide, vasoactive intestinal peptide (VIP), amylin or human pre-pro-NPY. Normal values were 20-80 pg/ml.

Leptin is an anorexigen agent and inflammatory marker and representing the quantity fat body store. Leptin was measured by radio-immuno assay (RIA) using a rabbit polyclonal antibody raised against purified recombinant human leptin (Linco Research, St. Louis, MO). The sensitivity limit is 0.5 ng/ml and linearity is 100 μg/l. Inter- and intra-

assay variation were 7.2 ± 0.4 and 25.6 ± 0.9 ng/ml, respectively; normal range is 1-7.8 ng/ml.

Gastrointestinal Peptides

The following peptides responsible for control of gastric acid secretion were measured by RIA.

GammmaDab [^{125}] gastrin was determined with a precipitating antiserum reagent to separate antibody-bound tracer from unbound tracer (INCSTAR Corp., Stillwater, MN). The sensitivity standard curve is defined as the smallest value that can be distinguished from zero. This value was calculated from the 95% confidence limit for 30 replicates at the zero point of the standard curve; calculated sensitivity is 6 pg/ml. Specificity is expressed as the ratio of gastrin concentration to the cross-reacting compound concentration at 50% inhibition of maximum binding (cross reactivity of gastrin G-17-I is 100%, gastrin G-17-II 64%, gastrin G-34-I 22%, gastrin G-13-I 29%, CCK 0.3% and secretin 0.3%). Normal range values were from 25-115 pg/ml.

CCK is an appetite modulator with orexigenic action. The 26-33 unsulfated cholecystokinin CCK fragment was determined (Peninsula, Belmont, CA,). Sensitivity is IC_{50}: 35 pg/100?l, specificity to CCK 26-33 and CCK 33 has 100% cross reactivity. Normal values were 12-20 pg/ml.

Pepsinogen I (Sorin Biomedica Diagnostics, Vercelli, Italy). Sensitivity is <1 ng/ml at 95% confidence limit. No significant cross reactions have been described at the highest concentrations present in serum samples for human- follicle stimulating hormone (hFSH), luteinizing hormone and thyroid-stimulating hormone, human gastrin, porcine glucagon, human-growth hormone (hGH)**,** proinsulin, insulin, C-peptide or prolactin. Normal values were 20-80 ng/ml.

Pepsinogen II (Sorin Biomedica Diagnostics). Specificity is 100%, and assay sensitivity is 0.3 ng/ml at 95% confidence limit. Normal values were 3-20 ng/ml. The pepsinogen I/pepsinogen II ratio is a non-invasive method to evaluate peptic secretion and functional gastric mucosal status. A high ratio (>6) indicates gastric hypersecretion [16].

Inflammatory Markers

C-reactive protein (CRP) was tested by nephelometric analysis (Boehring Dade., Germany), with a normal range <0.6 mg/dl.

TNF-α was measured using enzyme-amplified sensitive immunoassay (oligoclonal system with monoclonal antibodies, EASIA Medgenix Diagnostics, Belgium). The minimum detectable concentration (MDC) was estimated to be 3 pg/ml and was defined as the TNF-α concentration corresponding to the mean of 20 replicates of the zero standard ± 2 standard deviations. This method did not show cross-react with interleukin (IL), IL-1, IL-2 or interferon-α. Normal values ranged from 3-20 pg/ml. TNF-α has an anorexigenic effect.

IL-6 was measured using Easia (Medgenix Diagnostics), with a normal range of <0.5 pg/ml.

Venous occlusion test (VOT) was performed to stimulate endothelium, by inducing stasis in the right arm during 10 min by applying a sphygmomanometer cuff inflated to a pressure midway between systolic and diastolic values [mean arterial pressure = (systolic pressure + diastolic pressure)/2] [11].

Endothelial factors associated with endothelial fibrinolytic capacity included tissue-type plasminogen activator (t-PA) pre-VOT and post-VOT, (ELISA-plasma-t-PA antigenic coaliza t-PA: chromogenix, Mölndal, Sweden), and PAI (plasminogen activator inhibitor) levels determined by a commercial monoclonal ELISA (Tintelize, PAI Biopool, Umea, Sweden). We also calculated t-PA ratio (t-PA post-VOT/pre-VOT).

Endothelial damage markers were trombomodulin (Asserachrom TM: Diagnostica Stago, Asnières, France), endothelial relaxing factor nitric oxide (NO) measured as nitrate (NO_3) concentration pre-VOT and post-VOT by capillary electrophoresis. We also calculate NO_3-ratio: (post-VOT/pre-VOT).

Other cardiovascular (CV) risk markers included serum or plasma levels of fibrinogen (inflammatory and procoagulant marker) (thrombin time method described by Clauss), lipoprotein-a (Lp(a)) (sandwich-type enzyme-linked immunoassay, TintElize Lp(a); Biopool, Umea Swede), and homocysteine (Hcy) (high pressure liquid chromatography).

Blood samples were drawn in resting and fasting conditions, between 09:00h and 11:00h and collected into Vacutainer tubes (Becton Dickinson) containing 0.129 mol/L sodium citrate. The samples were centrifuged and tested immediately or stored at –70ºC until assayed.

Statistical analysis. Results are expressed as median and range values. Comparisons between data groups were performed using the non-parametric Mann-Whitney rank sum U test. Spearman's regression analysis and Student's *t* test were used for paired and non-paired data. A $p<0.05$ was considered statistically significant.

For security reasons, each variable was determined twice in groups II, III and controls. As results did not differ, we used and show the first value for comparisons.

RESULTS

Table **1** shows the values of the parameters and their differences among the four groups at baseline. Patients from group I showed significantly lower lymphocyte counts, prealbumin, IGF-I, transferrin, serum albumin, nPNA and Ccr than the remaining groups. These patients also showed higher TNF-α, IL-6 and CRP levels. CCK and pepsinogen I/II ratio were both higher in group I than group IV. These findings suggest an unusual situation of the *Hp*-infected patients with respect to the others. Renal creatinine clearance was significantly higher in group IV relative to group I (4.8 ± 1.6 *vs.* 2.7 ± 2.3 ml/min, $p<0.05$). The differences in the other two groups (4.7 ± 1.2 and 2.8 ± 0.9, respectively, for groups II and III) were not statistically significant. Serum Hb levels did not differ among groups (average 10.4-11.4 g/dl).

Table **2** shows changes in nutritional, inflammatory, appetite and GI markers in group I before and after *Hp* eradication. A significant increase was evident in nutritional mark-

Table 1. Nutritional, Inflammatory Markers and GI Peptides at Baseline

Parameters	Group I	Group II	Group III	Group IV
Diet assessment (kcal/kg/day)	22.9 ± 3.7 (a,b)	28.2 ± 4.7	24.8 ± 4.6 (a)	31.3 ± 1.8 (b)
Lymphocyte count(/mm^3)	1105 ± 259 (a,b)	1225 ± 230	1350 ± 62 (a)	1525±184 (b)
Prealbumin (mg/dl)	26.7 ± 6.4 (c,d)	32.7 ± 9.9 (c)	31.7 ± 0.5	36.1± 2.5 (d)
IGF-I (ng/ml)	151.8 ± 46 (e,f)	259 ± 91	216 ± 12 (e)	243 ± 40 (f)
Transferrin (mg/dl)	177.6 ± 27 (g,h)	242 ± 15 (g)	210 ± 47.7	233.4 ± 35 (h)
Cholesterol (mg/dl)	202 ± 45	227 ± 15.8	193 ± 8.8	215 ± 37
Serum albumin (g/dl)	3.4 ± 0.25 (i,j)	3.7 ± 0.13	3.7 ± 0.3 (i)	3.9 ± 0.15 (j)
Serum creatinine (mg/dl)	7.7 ± 2.3 (k)	7.78 ± 1.3	6.3 ± 1.4	6 ± 0.5 (k)
nPNA (g/kg/day)	0.9 ± 0.16 (i)	1.03 ± 0.1	0.89 ± 0.14	1.08 ± 0.1 (i)
C-reactive protein (mg/dl)	1.16 ± 1.14 (*)	0.7 ± 0.23	0.9 ± 0.6	0.6 ± 0.23 (*)
TNF-α (pg/ml)	127 ± 85.5 (m,n)	70.5 ± 25	60.5 ± 10 (m)	43.4 ± 5.4 (n)
IL-6 (pg/ml)	34.2 ± 18 (o,p,q)	13.4 ± 7 (o)	11.1 ± 7.9 (p)	1.02 ± 0.65 (q)
Leptin (ng/ml)	40.7 ± 23	35.2 ± 19	35.6 ± 14	43.4 ± 17
Gastrin (pg/ml)	206 ± 156	149 ± 59	136 ± 71	189 ± 36
Cholecystokinin (pg/ml)	47.8 ± 22 (r)	32.5 ± 17	36 ± 7.3	30.5 ± 6.2 (r)
Pepsinogen I (pg/ml)	183.5 ± 67	300 ± 90	196 ± 38.6 (s)	190.8 ± 41 (s)
Pepsinogen II (pg/ml)	25.4 ± 17 (t)	42.7 ± 19	42.1 ± 23	44.9 ± 12 (t)
Pepsinogen I/II ratio	7.3 ± 3.9 (v,w)	7.1 ± 4.7	4.6 ± 1.7 (v)	4.2 ± 3.4 (w)
Neuropeptide Y (pg/ml)	65.1 ± 27.1	66.3 ± 33	58.1 ± 9	69.2 ± 6.8

GI: gastrointestinal. IGF-I: Insulin growth factor type I. nPNA: normalized nitrogen-protein equivalence appearance TNF-α: tumor necrosis factor-alpha. IL-6: interleukin-6. Letters (a-w): statistically significant differences ($p<0.05$). (*): $p=0.054$.

ers, with decrease in acid GI hormone regulators and cytokines. In contrast, the four patients from group II showed no changes in nutritional markers (except a small increase in albumin; 3.7 ± 0.13 ± 3.9 ± 0.3 g/dl, $p<0.01$) after *Hp* eradication. Daily food intake, lymphocyte count, prealbumin, IGF-I and transferrin showed no significant changes. These results may be influenced by the small size of this series. As a result of *Hp* treatment, however, cytokine levels except CRP (0.7 ± 0.23 vs. 0.6 ± 0.3 mg/dl, NS) showed a significant decrease after *Hp* eradication (TNF-α, 70.5 ± 25.3 vs. 41.3 ± 8.6 pg/ml, $p<0.01$ and IL-6, 13.4 ± 7 *vs.* 3.9 ± 4.6 pg/ml, $p<0.01$). Relative to appetite modulator peptide changes, NPY also increased (66.3 ± 34 *vs.* 83.4 ± 43 pg/ml, $p<0.05$), although CCK and leptin did not show modifications (32.5 ± 17.2 *vs.* 25.7 ± 8 pg/ml and 35.2 ± 19.7 *vs.* 25 ± 13 ng/ml, NS, respectively). Similarly, GI

Table 2. Changes in Nutritional, Inflammatory Markers and GI Peptides Prior and Post -*Hp* Eradication (Group I)

Parameters	Pre- eradication	Post- eradication	p
Diet assessment (kcal/kg/day)	22.9 ± 3.7	29.4 ± 4.4	<0.01
Lymphocyte count (/mm^3)	1105 ± 259.4	1330.8 ± 316	<0.05
nPNA (g/kg/day)	0.9 ± 0.16	1.07 ± 0.3	<0.05
Prealbumin (mg/dl)	26.7 ± 6.5	33.9 ± 56.6	<0.01
IGF-I (ng/ml)	151.8 ± 46	225 ± 111.4	<0.05
Albumin (g/dl)	3.48 ± 0.3	3.67 ± 0.35	<0.05
C-reactive protein (mg/dl)	1.16 ± 1.14	0.88 ± 1.2	<0.1(NS)
TNF-α (pg/ml)	127 ± 85.5	44.4 ± 29.3	<0.01
IL-6 (pg/ml)	34.2 ± 18.4	11.3 ± 9.8	<0.001
Leptin (ng/ml)	40.7 ± 23	27 ± 14.5	<0.01
Neuropeptide Y (pg/ml)	65 ± 27.5	91.3 ± 25.7	<0.01
Cholecystokinin (pg/ml)	47.8 ± 22.4	25.7 ± 11.5	<0.01
Gastrin (pg/ml)	206 ± 156	153 ±45	<0.1(NS)
Pepsinogen I/II ratio	7.3 ± 3.9	4.1 ± 2.2	<0.05

GI: gastrointestinal. nPNA: normalized nitrogen-protein equivalence appearing. IGF-I: insulin growth factor. TNF-α: tumor necrosis factor. IL-6: interleukin-6.

Table 3. Changes in Endothelial Function and Damage Markers Before and After *Hp* Eradication (Group I)

Parameters	Normal range	Prior-eradication	Post- eradication	p
CCr (ml/min)	90-110	2.7 ± 2.3	2 ± 2.4	NS
PAI (ng/mL)	<10	13.2 ± 2.8	9 ± 3.2	<0.05
tPA-pre VOT (ng/mL)	1-12	10.7 ± 6.1	9.1 ± 5.2	NS
tPA-post VOT (ng/mL)	variable	16.4 ± 3.3	18.6 ± 5.4	<0.05
tPA-ratio	>2	1.5 ± 0.5	2 ± 1	<0.05
NO_3-pre VOT (μmol/L)	40-60	54.3 ± 11.1	42.2 ± 14.1	=0.07(NS)
NO_3-post VOT (μmol/L)	variable	55 ± 18.4	58.1 ± 17	NS
NO_3-ratio	variable	1.01 ± 1.6	1.38 ± 1.2	<0.05
TM (ng/mL)	14-55	313.1 ± 51	270 ± 38	<0.05
Lp(a) (ng/mL)	<20	38.4 ± 18	29 ± 9.4	<0.05
Hcy (ng/mL)	5-10	33 ± 17.6	28 ± 11.2	NS

PAI: plasminogen activation inhibitor. tPA: tissue-type plasminogen activator. NO_3: nitrate. TM: Thrombomodulin. Lp(a): lipoprotein (a). Hcy: homocysteine. Ccr: creatinine clearance. VOT: Venous occlusion test.

peptides, gastrin, CCK, pepsinogen I and II and the pepsinogen I/II ratio showed no changes. The *Hp* breath test nonetheless is superior in accuracy to all of these tests in confirming *Hp* eradication.

Table **3** shows changes in endothelial function and damage markers prior and after *Hp*-eradication. We found an increase in tPA-ratio and NO_3-ratio, and decrease in PAI, Lp(a) and TM.

The pre- and post-*Hp* eradication results in healthy control individuals are shown in Table **4**. A remarkably lower effect was registered in inflammatory values, which did not reach statistical significance, except for IL-6. In contrast, GI

Table 4. Changes in Nutritional, Inflammatory Markers and GI Peptides in the Non-uremic Patients (Control Group), Prior and Post *Hp*-Eradication

Parameters	Prior-eradication	Post-eradication	p
Lymphocyte count (/mm^3)	2105 ± 178	2231 ± 323	NS
Prealbumin (mg/dl)	39 ± 5.4	41 ± 3.3	NS
IGF-I (ng/ml)	232 ± 24	245 ± 41	NS
Albumin (g/dl)	4.3 ± 0.8	4.41 ± 0.5	NS
C-reactive protein (mg/dl)	1.11 ± 0.75	0.44 ± 0.26	<0.1(NS)
TNF-α (pg/ml)	17.6 ± 7.3	12 ± 6.5	<0.1(NS)
IL-6 (pg/ml)	3.8 ± 2.01	1.71 ± 1.16	<0.05
Leptin (ng/ml)	5.38 ± 2.3	6 ± 2.5	NS
NPY (pg/ml)	48 ± 22.4	56 ± 32.2	NS
CCK (pg/ml)	25.7 ± 8.4	13.7 ± 6.3	<0.05
Gastrin (pg/ml)	153 ± 38	98 ± 22.1	<0.01
Pepsinogen I/II ratio	7.7 ± 2.2	4.3 ± 1.5	<0.05
PAI (ng/mL)	10.1 ± 2.3	7.8 ± 2.4	<0.05
tPA-ratio	2.8 ± 1.1	3 ± 1.5	NS
NO_3-ratio	1.2 ± 0.3	1.34 ± 0.5	NS
TM (ng/mL)	46 ± 8.8	36 ± 12	NS
Lp(a) (ng/mL)	14 ± 6.1	10.7 ± 4.6	=0.07(NS)
Hcy (ng/mL)	7.7 ± 3.1	6 ± 2.1	NS

GI: gastrointestinal, IGF-I: insulin growth factor. TNF-α: tumor necrosis factor. IL-6: interleukin 6. NPY: neuropeptide Y. CCK: cholecystokinin. PAI: plasminogen activation inhibitor. tPA: tissue-type plasminogen activator. NO_3: nitrate. TM: Thrombomodulin. Lp(a): lipoprotein (a). Hcy: homocysteine.

parameters had similar effects (decrease in CCK, gastrin and pepsinogen I/II-ratio), demonstrating the positive effect of the treatment. The lack of effect on nutritional parameters establishes differences with similarly infected and treated uremic subjects. No patients from the control group suffered anorexia, but five experienced dyspepsia. In regard to endothelial function, PAI decreased after *Hp*-eradication.

Table **5** shows the different statistically significant linear correlation coefficients among the parameters studied at baseline.

DISCUSSION

The most important finding in this study is that *Hp* infection is associated with anorexia and malnutrition in PD patients, and that its eradication has effects on both conditions. The mechanism through which *Hp* induces these complications may be cytokine overproduction, with cachectic action accentuated by retention due to uremic status. Residual renal function (RRF) appears to play a crucial role in the preservation of appetite. *Hp* eradication was associated with nutritional status and appetite improvement. *Hp* infection increased gastric acid secretion, as estimated by GI peptide serum levels.

***Hp* infection association with cytokine overproduction**. High plasma levels of TNF-α, IL-1, IL-6 and other pro-inflammatory substances have been described in the non-uremic population infected by *Hp* [5, 6, 17]. A high serum concentration of cytokines has been found in dialysis patients [11, 12, 18). In patients from group I, the higher cytokine concentration thus appears to be due to simultaneous *Hp* infection and uremia.

Deleterious effects of cytokines in uremic and non-uremic subjects. We reported an association between high TNF-α plasma levels and anorexia in PD patients [11]. Levels of TNF-α greater than 65 pg/ml were related to deleterious effects including anorexia, acidosis, hypertriglyceridemia, malnutrition, anemia (recombinant human erythropoietin resistance) and uremic neuropathy [12]. Other investigators found pathogenic association between TNF-α and muscular structural protein breakdown *via* the ubiquin-proteases system, inhibition of hepatic albumin synthesis and renal amyloidosis [3, 4]. We therefore propose that TNF-α should be considered a uremic toxin.

The results showing a negative association between TNF-α and IL-6 with nutritional markers including daily food intake support these findings. Experiments in animals dem-

Table 5. Linear Regression Coefficients for Studied Variables at Baseline

Variable	Albumin	Pre-albumin	Transferrin	Cholesterol	Daily food intake	TNF-α	Gastrin
Albumin		0.43 **		0.38 *	0.39 *	-0.4 *	
Prealbumin	0.43 **				0.39 *	-0.46 **	
Cholesterol	0.38 *						
nPNA		0.35 *					
IGF-I			0.4 *			-0.38 *	
Daily food intake	0.39 *	0.39 *				-0.55 **	
IL-6	-0.36 *	-0.34*	-0.38 *	-0.48 **		0.44 *	
C-PR	-0.35 *	-0.36 *				0.68***	
NPY	0.41 *	0.48 **			0.53 **	-0.6 ***	
CCK	-0.38 *			-0.35 *	-0.48**		-0.43 **
Pep-I							0.41 *
Pep I/II						0.33*	0.48 **
PAI						-0.5**	
tPA-ratio						0.46*	
NO_3-ratio	0.36						
TM		0.41*				0.33*	
Lp(a)						0.4*	
Hcy	0.38*						

IGF-I: insulin growth factor. TNF-α: tumor necrosis factor. IL-6: interleukin 6. NPY: neuropeptide Y. CCK: cholecystokinin. Pep.: Pepsinogen. PAI: plasminogen activation inhibitor. tPA: tissue-type plasminogen activator. NO_3: nitrate. TM: Thrombomodulin. Lp(a): lipoprotein (a). Hcy: homocysteine.
*: <0.05. **: <0.01. ***: <0.001. The non-expressed coefficients are not statistically significant.

onstrated that intracerebral r-mTNF-α injection induces anorexia, loss weight and cachexia [19, 20].

The results in Table **1** show clear clinical evidence for the participation of TNF-α and IL-6 in decreased food intake (uremic anorexia pathogenic factor). We recently proposed a "tryptophan-serotonin" hypothesis [21] to explain appetite loss in dialysis patients, in which inflammatory mediators play an important pathogenic role.

In the same study [11], patients with GI symptoms showed high TNF-α plasma levels, and evidence was found for RRF participation in protection and preservation of appetite; the present results confirm these observations (Table **1**). Moreover, RRF appears more important than peritoneal urea-weekly-KT/V in the preservation of normal food intake. We speculate that patients with RRF are protected from the effects of chronic and silent infections such as *Hp*. Low creatinine clearance may be sufficient to eliminate an important part of low molecular weight pro-inflammatory cytokines such as TNF-α (55 KD) [22]. The CANUSA study [23] showed that RRF protects dialysis patients from morbidity and mortality. The decrease in cytokine levels and the parallel increase in nutritional markers after *Hp* eradication was associated to a greater RRF.

Our findings support to the inflammatory hypothesis as a cause of malnutrition [1]. It may be that dialysis patients suffer these inflammatory processes in a cyclic manner, in direct proportion to time on dialysis and in inverse proportion to RRF. Silent inflammation of organs such as heart, GI tract, liver or blood vessels [11, 17] could be a source of cytokine overproduction; in such cases, an increase in dialysis dose would not improve the situation.

Another mechanism by which *Hp* infection may induce loss of appetite is by decreasing GI motility [24], possibly also mediated by TNF-α [22, 25]. Low GI motility is a frequent finding in dialysis patients [26].

CCK and leptin, both anorexigenic substances, and NPY, the most potent orexigen known, are important biological appetite modulators that are also affected by uremia [11, 27]; association between inflammatory mediators and leptin has been described [27]. After *Hp* eradication in group I, CCK and leptin decrease and NPY increases (Table **2**). A simultaneous increase appeared in daily food intake and improvement in nutritional markers. The key factor to understanding

the decrease in CCK plasma levels after treatment may be inherent to a gastric acid-stimulating hormone cycle disorder, frequently found in non-uremic *Hp* infected patients [28, 29]. Gastrin and pepsinogen levels decreased in our patients after *Hp* eradication (Table **2**).

In normal conditions, dietary proteins stimulate antral G cells to release gastrin to blood; released gastrin stimulates stomach parietal cells, increasing the intraluminal acid secretion mediated by gastrin releasing factor. The low antral pH results in negative feedback regulated by somatostatin (D cells) and CCK, both neutralizing the pH of the alimentary bolus and protecting the duodenum from low pH [29]. In the presence of *Hp* this feedback is broken, however, and CCK, gastrin, somatostatin and pepsinogens show parallelism, increasing or decreasing simultaneously [30]. Moreover, these hormones are markedly elevated in dialysis patients and normal feedback may be lost. The increase in stomach acid secretion may contribute to anatomical abnormalities in the GI tract.

One of the most important markers of gastric acid secretion is the pepsinogen I/II ratio. High pepsinogen I/II ratio is associated to high acid secretion [16]. *Hp* eradication decreased all gastric acid mediators in all affected patients (Tables II, IV). In *Hp*-infected control patients, we found a decrease in gastric acid mediators (gastrin, pepsinogens and CCK) (Table **4**), which were previously described after *Hp* eradication [28, 29].

Cytokines also participate in gastric acid secretion. The antral of IL-1 and TNF-α concentrations are thus inversely related to somatostatin concentration [31]. One could speculate on a similar effect on CCK and pepsinogen levels.

The changes in NPY after *Hp* eradication (Table **2**) can not be explained by the gastric acid secretion cycle. However, an influence of TNF-α on NPY could not be discarded. Recently, an inhibitory effect of TNF-α on a neuropeptide with a structure similar to that of NPY was described [32]. Supporting this idea are the results showed here and in other study performed by our group [11], where we found a negative linear correlation between both substances. Moreover, the decrease in inflammatory markers was proportionally inverse to the increase in NPY levels (Table **2**).

According to these results, the strong inverse correlation between TNF-α and IL-6 levels with nutritional markers including daily food intake may indicate that these cytokines are better inflammatory markers than CRP. Furthermore, IL-6 is a stimulator of CRP synthesis [33].

Endothelial Function, Inflammation and *Hp*-Eradication

tPA is an endothelium-derived fibrinolytic glycoprotein released by exercise, VOT and stimulation with desmopresin. tPA values after stimulation by VOT (expressed as tPA-ratio), indicates an adequate fibrinolytic capacity [34-36]. It has been demonstrated that patients suffering severe atherosclerosis or unstable angina show low tPA levels after stimulation [37]. Uremia *per se* is associated with poor tPA response for unknown causes [34, 35]. Importantly, we have found a clear association between elevated pro-inflammatory molecules (TNF-α) and a decrease in tPA-ratio (Table **5**).

Experimentally, the stimulation of human endothelial cells from umbilical vein endothelial culture with IL-1 and TNF-α, reduces tPA synthesis [38]. Since tPA can also be considered as a pro-inflammatory molecule [36], the spontaneous or post-VOT elevation of tPA may result in decrease of tPA-ratio and increase in PAI levels, predisposing to coagulant phenomena [34-36]. Effectively, we have found that *Hp*-eradication with decrease in inflammatory markers was associated with increase in tPA-ratio (Table **3**). Chia S, *et al.* [39], found an increase in spontaneous tPA after systemic inflammation and concluded, that SI invoke a protective response mediated by enhancing endothelial fibrinolytic capacity.

Another important fibrinolytic inhibitor, the PAI, was augmented by inflammation. It has been demonstrated *in vivo* and *in vitro* that cytokines (IL-1 and TNF-α) induce elevation of PAI [40, 41]. Moreover, malnourished and inflamed patients, show hepatic acute hyperproduction of half-life proteins such as C-reactant protein, prealbumin, fibronectin, PAI and others [36]. Finally, and according with our results and shown by a longer series [42], the elevation of PAI associated with inflammation should be considered as a cardiovascular risk factor due to predisposition to thrombotic events.

Endothelial damage as a consequence of injuries is followed by TM and NO release [35, 41-44]. TM is a structural protein from the endothelial cells membrane, released in injury conditions, which represents the dead cells burden [36, 41]. Due to its renal excretion, high plasma levels are found in uremia [35, 44]. TM released in plasma activates protein-C to inhibit fibrin formation [41]. TM plasma levels are also affected by inflammatory molecules, proteolysis and oxidative stress. In culture cells, the stimulation with high doses of TNF-α induces a dramatic increase in TM [36, 40]. Our results confirm that after *Hp*-eradication TM decreased, representing and improving in endothelial structure. NO is the most potent vasodilator synthesized by endothelial cells, able to inhibit platelet adhesion, release mitogenic factors, and cause proliferation of muscle vessel cells [45]. Deficient NO production and early atherosclerosis [46] have been found after administration of recombinant TNF-α. Moreover, in endothelial cell culture, TNF-α administration reduces the half-life of mRNA encoding for NO-synthase [47, 48], predisposing to NO-depend vasoconstriction [49]. Effectively, after *Hp*-eradication, patients showed spontaneous decrease in NO_3-pre VOT and in consequence a decrease in NO_3-ratio (VOT, NO_3-post/-pre) (Table **3**).

Lipoprotein$_{(a)}$, Fibrinogen and homocysteine are recognized **CV risk factors** [50] frequently elevated in dialysis patients [40, 50]. Inflammation causes increase in the hepatic synthesis acute phase reactants. Lp(a) is one of these proteins able to induce procoagulable status [51]. Fibrinogen and Hcy may also be affected by inflammation [40, 41, 50]. However, we did not find differences in both parameters after *HP*-eradication (Table **3**). Fibrinogen showed a not statistically significant mild decrease. Recently, it has been described that fibrinogen and other pro-inflammatory parameters were important predictor of cardiac ischemic attack in patients infected by *HP* (78.3% *vs.* controls 56.2%, $p<0.05$) [52]. The same association was found by Pellicano R *et al.* [53].

They investigated the association between unstable angina and anti-*HP* seropositivity in 32 patients, which was matched with control group (n=62). Again 81% of studied patients showed *HP* IgG antibodies vs. 53%, supporting the hypothesis that a chronic and silent inflammation is able to triggering pro-coagulant, vasoconstrictor and possibly vessel proliferating phenomena.

Finally, diagnosis of *Hp*-infection acquires new importance in the evaluation of dialysis patients with MIA syndrome, since *Hp* eradication may be a key in the management of malnourished patients. We believe that diagnosis and treatment of *Hp* infection should precede other approaches to malnutrition.

In conclusion, *Hp* infection in peritoneal dialysis patients causes cytokine overproduction, which is further exacerbated by poor renal elimination. Cytokines induce anorexia, cachexia, malnutrition and endothelial dysfunction. In consequence, residual renal function plays a critical role in protection from extending the period of high cytokine levels and may thus contribute to the preservation of the nutrition status and vascular integrity in PD patients. The changes associated with *Hp* eradication supports the role of the MIA syndrome pathway in dialysis patients.

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True Deficiency of Antioxidant Vitamins˚E and A in Dialysis Patients. Relationship with Clinical Patterns of Atherosclerosis

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Atherosclerosis is an important cause of morbidity and mortality in peritoneal dialysis (PD) patients. Oxidative stress plays a role in the pathogenesis of uremic atherosclerosis. Although antioxidant substances (vitamins˚A and E) are elevated in the plasma of dialysis patients, intracellular and clinical signs of hypovitaminosis are frequently found. Recently, the importance of vitamin/carrier complexes as a marker of vitamin bioavailability has been demonstrated. In the present study, we analyzed vitamin˚A and E bioavailability, measured as vitamin/carrier complexes, and the relationship of those measurements with clinical atherosclerosis status in PD patients.

We studied 45˚patients (15˚men, 30˚women), who were divided into four groups according to clinical atherosclerotic score (CAS). Five cases were scored as CAS grade˚1 (low CAS); 9 as CAS-2; 18 as CAS-3; and 13 as CAS-4. Vitamins˚A and E and their carriers [prealbumin and retinol binding protein (vitamin˚A), and cholesterol and triglycerides (vitamin˚E)] were determined.

Plasma levels of vitamin˚A were low in 5˚patients, normal in 7˚patients, and high in 33˚patients. By correcting the values for the carrier levels, we created three groups: 24˚patients showed low vitamin˚A/carrier complex (5 from the low plasma vitamin˚A group, 6 from the normal-value group, and 13 from the high-value group); 11 patients were in the group with normal vitamin˚A/carrier (1 from the normal plasma vitamin˚A group, and 10 from the high-value group); and 10˚patients were in the group with high vitamin˚A/carrier. The vitamin˚A/carrier complex showed a statistically significant, negative linear correlation with CAS and with serum iron.

Low vitamin˚E plasma levels were found in 1˚patient, normal levels in 28˚patients, and high levels in 16˚patients. When those values were corrected using the carrier values, three groups were also created. The group with low vitamin˚E/carrier complex contained 24˚patients (1 from the low-value group, 22˚from the normal-value group, and 1 from the high-value group). The group with normal vitamin˚E/carrier complex contained 21˚patients (15˚from the group with high vitamin˚E values, and 6 from the normal-value group). By univariate logistic regression analysis, significant associations between CAS and vitamin˚E plasma levels, vitamin˚E/carrier, age, and serum albumin were found. In the multiple logistic regression analysis, we confirmed that vitamin˚E/carrier complex, age, and serum albumin showed independent associations with CAS, but not with vitamin-only plasma levels.

Our results in PD patients show a vitamin/carrier complex disorder that results in elevated vitamin mobilization from pool and target cells. Our results and the findings of other researchers about intracellular vitamin˚A and E deficiencies may change the traditional concept of hypervitaminosis˚A and E in uremic patients.

Key words
Vitamin˚A, vitamin˚E, vitamin/carrier complex, antioxidants, atherosclerosis

Introduction

Reactive oxygen species are factors commonly implicated in the development of tissue injury in renal disorders and in dialysis patients. Antioxidant vitamins decrease tissue damage by trapping organic free radicals and inhibiting lipid peroxidation. Neverthe-

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less, supplementation therapy with vitamin˚A and E is not recommended in such patients, owing to increased serum vitamin levels.

Vitamin˚A and E deficiencies have been implicated in premature development of atherosclerosis (1). Accelerated atherosclerosis and cardiovascular (CV) complications are recognized as major survival-determining factors for long-term maintenance dialysis patients (2,3). In peritoneal dialysis (PD) patients, the complexity of the situation is increased, because the atherosclerotic process includes specific factors such as hyperglycemia, hyperlipidemia, hyperinsulinemia, obesity, and hypoalbuminemia. In addition, signs of endothelial dysfunction are frequently found in uremic patients (3,4). The adoption of strategies to avoid endothelial injury are therefore relevant.

Increasing plasma levels of antioxidative agents should improve the hyperoxidative status of uremia. Nonetheless, disorders in vitamin˚A and E intracellular metabolism, bioavailability, membrane receptor, and vitamin/carrier complex have been suggested in uremic patients (1). For instance, plasma levels of vitamin˚A and its carrier are both increased with respect to skin content of vitamin˚A. In spite of the increased plasma levels, the vitamin˚A pool in the skin is not increased, and cutaneous lesions resembling the abnormalities found in hypovitaminosis˚A (xerosis) are present in uremia. It has therefore been suggested that, in uremia, an alteration occurs in cutaneous vitamin˚A receptor or in the vitamin˚A/carrier complex, with reduction of vitamin entry into cells (1,5).

A similar situation may arise with vitamin˚E status in dialysis patients, induced by an increase in the vitamin˚E carrier (cholesterol and triglycerides) level. Vitamin˚E is a scavenger, largely localized within membranes. Because of its high lipid solubility, it plays a special role with regard to the critical membrane lipid targets of free radicals (6). Uremic hyperlipidemia and antioxidant deficiency favoring oxidative stress is an added factor favoring development of atherosclerosis (7). In addition, recent research demonstrates the importance of measuring vitamin/carrier complex as a marker of vitamin bioavailability (8).

Our aim was to study vitamin˚A and E bioavailability, measured as vitamin/carrier complex, and the relationship of those measurements with clinical atherosclerosis status in PD patients.

Patients and methods

We studied 45˚clinically stable PD patients. The group included 15˚men and 30˚women, ranging in age from 27˚to 86˚years (mean: 51.8± 13.9˚years); 26 on continuous ambulatory peritoneal dialysis (CAPD), and 19˚on automated peritoneal dialysis (APD) [14˚on continuous cycling peritoneal dialysis (CCPD), and 5 on nightly peritoneal dialysis (NPD)]. The mean period on PD was 33.5˚± 37˚months (range: 1˚—179˚months). No acute or chronic active disorders were present during the 6˚months prior to the study. The causes of renal failure were glomerulonephritis (10˚cases), diabetes (8˚cases), polycystic kidney disease (7˚cases), chronic pyelonephritis (5˚cases), nephrosclerosis (5˚cases), unknown etiology (4˚cases), systemic disease (presently inactive, 4˚cases), and other causes (2˚cases). Patients with cirrhosis, thyroid disease, and familiar dyslipidemias were excluded.

The patients were divided into four groups according to clinical atherosclerosis score (CAS) (9). Five cases were scored CAS grade˚1 (lowest atherosclerotic status); 9 were scored CAS-2; 18 were scored CAS-3; and 13 were scored CAS-4.

Prior to starting PD, 32˚patients were diagnosed with high blood pressure (HBP); however, only 28 had HBP when the present study was performed. Of the 28, 7 were using angiotensin-converting enzyme inhibitors; 4, calcium-channel blockers; 7, other drugs (α and β adrenergic blockers, direct vasodilators, and angiotensin˚II—receptor blockers); and 10, combinations of antihypertensive drugs. Lipid-lowering agents were withdrawn 15˚days before the study. By echocardiogram, 38˚patients showed left ventricular hypertrophy, 6 in severe grade. Low-dose aspirin was used by 8˚patients; 36˚patients were physically active; and 5˚patients were active smokers.

Dialysis adequacy

Dialysis adequacy was assessed by weekly urea Kt/V and normalized protein catabolic rate (nPCR).

Antioxidants

Antioxidant levels were determined in fasting conditions. Serum albumin was measured by the colorimetric method (Hitachi 704: Boehringer Mannheim, Mannheim, Germany); transferrin, ceruloplasmin, and vitamin˚A carriers [prealbumin and retinol binding protein (RBP)] by the immunonephelometric method

(Behring Nephelometer Terminal S.A.: Behringwerte AG, Marbus, Germany); and bilirubin, glucose, and uric acid by Hitachi 704. Vitamin˚A and E plasma levels were analyzed using high-performance liquid chromatography (HPLC). The HPLC instrument used was from Waters Associates, Inc. (Milford, MA, U.S.A.), and consisted of a 600E solvent delivery system, an automatic sample injector model 712˚WISP, and a model 481˚Lambda Max spectrophotometer programmable multi-wavelength detector injector with a Millennium data station. Vitamins were separated on a 3.9↔150˚mm resolve C18˚column (Waters Associates) packed with 5˚µ particles. The solvent was methanol:water (95:5) at a flow rate of 1.2˚mL/min. Vitamins were detected at 290˚mm. For the analysis, a 100˚µL blood serum sample and 100˚µL of an internal standard retinol acetate solution in ethanol (1˚µ/mL) were mixed on a vortex mixer for 10˚seconds. For lipid extraction, 200˚µL of spectrograde hexane was added, and the contents were mixed for 60˚seconds. Tubes were centrifuged (2000g for 5˚minutes) to separate the phases, and then the solvents were transferred to another tube and evaluated in an automatic environment (Speed-Vac: Savant Instruments, Farmingdale, NY, U.S.A.). The lipids were dissolved in 200˚µL methanol, and approximately 50˚µL of the solution was injected into the chromatograph. Normal values in healthy populations are 8˚— 21˚µg/mL α-tocopherol (vitamin˚E plasma levels) and 0.4˚— 0.8˚µg/mL retinol (vitamin˚A plasma levels).

Vitamin˚A carrier

Prealbumin and RBP were determined by an immunonephelometric method (BN-100: Behringwerke, Marburg, Germany). Reference values in healthy populations are 10˚— 40˚mg/dL for prealbumin and 3˚— 6˚mg/dL for RBP.

Vitamin˚E carrier

Cholesterol was measured by the colorimetric method, and triglycerides by the Hitachi 704. Normal ranges are 150˚— 250˚mg/dL for cholesterol and 50˚— 175˚mg/dL for triglycerides.

Vitamin/carrier complexes

Vitamin˚A/carrier and vitamin˚E/carrier complexes (µg/mg) were calculated using these equations:

vitamin˚A serum levels (µg/mL)˚/ [˚RBP (µg/mL)˚↔ 7.342]

vitamin˚E serum levels (µg/mL)˚/ [cholesterol (mg/mL)˚+ triglycerides (mg/mL)]

Normal values for vitamin/carrier complexes were obtained from a selected group of healthy individuals (without dyslipidemias): 30˚women and 40˚men, ranging in age from 25˚— 50˚years. The normal range for vitamin˚A/carrier complex was 0.8˚— 1.2˚µg/mg; for vitamin˚E/carrier complex, it was 6˚— 16˚µg/mg.

Statistical analysis

Comparisons between groups of data were performed using the Student *t*-test and the Mann—Whitney *U*-test. We also used the linear Spearman regression analysis. A *p*˚value less than 0.05 was considered statistically significant. A multivariate analysis (logistic regression) was performed to analyze the association between the dependent variable (severity of CAS, high or low) and the independent variables, vitamin˚E/carrier (continuous), age (continuous), and serum albumin (continuous). All variables were analyzed individually against the dependent variable, and multiple regression analysis was then performed. For CAS and multivariate analysis, all patients were grouped as low score (CAS-1 and -2) or high score (CAS-3 and -4). Results are given as median and range.

Results

Table˚I shows the general analytical data.

Vitamin˚A plasma levels were low in 5˚patients, normal in 7˚patients, and high in 33˚patients. By correcting for carrier levels, we created three groups: 24˚patients showed low vitamin˚A/carrier complex (5˚from the low plasma vitamin˚A group, 6 from the normal-value group, and 13˚from the high-value group). The group with normal values of vitamin˚A/carrier contained 11 patients (1 from the normal-value vitamin˚A group, and 10 from the high-value group). Finally, 10 patients were in the group with high vitamin˚A/carrier levels, all from the high plasma value group.

The vitamin˚A/carrier complex showed a statistically significant, negative linear correlation with CAS (r˚= —0.36 p˚< 0.05) and with serum iron (r˚= —0.34 p˚< 0.05).

With respect to vitamin˚E plasma levels, we found low values in 1˚patient, normal values in 28˚patients, and high values in 16˚patients. When those values were corrected for the carrier values, three groups also

TABLE I General analytical parameters

Parameters	*Measured value*[a]	*Normal range*
Hemoglobin (g/dL)	10.9±1.4	10—15
Urea (mg/L)	144.8±37	Variable
Albumin (g/dL)	3.79±0.49	3.8—4.5
Prealbumin (mg/dL)	32.3±9.3	>30[b]
RBP (mg/dL)	12±4.6	3—6
Cholesterol (mg/dL)	226±47.8	150—250
Triglycerides (mg/dL)	153±74	50—175
Creatinine (mg/dL)	9.4±2.2	Variable
CCr (mL/min)	2±2.2	Variable
Weekly urea Kt/V	2.2±0.46	>2
nPCR (g/kg daily)	1±0.24	>1
Iron (µg/dL)	62±22.7	50—145
Ferritin (ng/mL)	267±224	50—250
Transferrin (mg/dL)	251±50	209—389
Uric acid (mg/dL)	5.8±3.6	5—8
Total bilirubin (mg/dL)	0.8±0.3	<1.5
Vitamin˚A (µg/mL)	1.36±0.77	0.4—0.8
Vitamin˚E (µg/mL)	20.3±8.1	8—21
Vitamin˚A/carrier (µg/mg)	0.86±0.44	0.8—1.2
Vitamin˚E/carrier (µg/mg)	6.44±2.8	6—16

[a] All values mean˚± standard deviation.
[b] Accepted value in uremic status.
RBP˚= retinol binding protein; CCr˚= creatinine clearance; nPCR˚= normalized protein catabolic rate.

emerged. The group with low vitamin˚E/carrier complex contained 24˚patients (1 from the low plasma level group, 22˚from the normal-value group, and 1 from the high-value group). The group with normal vitamin˚E/carrier complex had 21˚patients (15˚from the group with high vitamin˚E plasma levels, and 6 from the normal-value group). No patient showed high vitamin˚E/carrier complex values. Vitamin˚E/carrier complex showed an inverse correlation with CAS (r˚= —0.49p˚< 0.01) and with age (r˚= —0.34p˚< 0.05). Women showed significantly higher vitamin˚E plasma levels than did men [6.9˚± 3.2˚µg/mL (n˚=30) vs. 5.5˚± 1.6˚µg/mL (n˚= 6), p˚< 0.05].

Vitamin˚Aand E plasma levels showed significant linear correlation with other antioxidant substances. Vitamin˚A was correlated with transferrin (r˚= 0.36, p˚< 0.05), ceruloplasmin (r˚= 0.3, p˚< 0.05), uric acid (r˚= 0.4, p˚< 0.01), total bilirubin (r˚= 0.35, p˚< 0.05), glucose (r˚= 0.38, p˚< 0.05), vitamin˚E (r˚= 0.36, p˚< 0.05), vitamin˚E/carrier (r˚= 0.74, p˚< 0.01), and albumin [r˚= 0.28, p˚= 0.054 (NS)]. Vitamin˚E was correlated with transferrin (r˚= 0.4, p˚< 0.05), ceruloplasmin (r˚= 0.38, p˚< 0.05), uric acid (r˚= 0.38, p˚< 0.05), and albumin (r˚= 0.33, p˚< 0.05).

Using multivariate analysis, no significant associations were found between plasma levels of vitamin˚A or vitamin˚A/carrier complex and the remaining variables studied.

By univariate logistic regression analysis, significant associations were found between CAS and vitamin˚E plasma levels [odds ratio (OR): 0.86; 95% confidence interval (CI): 0.77˚to 0.95; p˚= 0.04], vitamin˚E/carrier (OR: 0.67; 95% CI: 0.5˚to 0.9; p˚= 0.01), age (OR: 1.13; 95% CI: 1.04˚to 1.23; p˚< 0.05), and serum albumin (OR: 0.086; 95% CI: 0.013˚to 0.54; p˚= 0.01).

By multiple logistic regression analysis, we confirmed that vitamin˚E/carrier complex (OR: —0.63; 95% CI: 0.52˚— 0.8p˚= 0.04), age (OR: 1.38; 95% CI: 1.14˚— 1.5p˚= 0.04), and serum albumin showed an independent association with CAS (OR: —0.4; 95% CI: 0.018˚— 0.82p˚= 0.01).

Discussion

High plasma levels of vitamins˚A and E have traditionally been found in uremic patients (1). This apparent hypervitaminosis is caused by uremic retention (1,2,10). Evidence is increasing that, due to their antioxidant effects, those vitamins and vitamin˚C protect against a number degenerative disorders, including CV disease, cataracts, and certain types of cancer (11,12).

Uremia is considered a pro-oxidative state (8), manifested in part by accelerated atherosclerosis, which determines survival in long-term maintenance dialysis patients (1,2). Oxidative stress is a complex process implicated in endothelial cell injury and dysfunction (3,4). Several longitudinal and cohort studies have examined the relationship between antioxidant intake and risk of CV events. Patients with angina pectoris showed lower plasma vitamin˚E concentrations than did normal subjects (13). Hypervitaminosis˚A in hemodialysis (HD) patients has been associated with anemia and hypercalcemia. Some cases of true vitamin˚Atoxicity have also been reported in such patients (14). Because adequate vitamin˚A intake is covered by the renal diet, no additional supplementation is recommended; supplementation is, in fact, contraindicated. Nonetheless, clinical signs of vitamin˚A and E deficiency, with lower tissue levels, have been described in uremic patients (1,5,6). Vitamin/carrier complexes have recently been described as powerful

indicators of intracellular vitamin deficiency in children with cystic fibrosis (8).

Our results showed that 13 of 33˚patients with elevated vitamin˚A plasma levels (39.4%) showed lower levels after correction for transport. In addition, 15 of 16˚patients with high vitamin˚E plasma levels (93.7%) showed normal corrected values, and none showed high corrected values. Those data confirm that dialysis patients with normal or high vitamin plasma levels may have a real deficiency, as demonstrated by measurement of the carrier complex. That situation could increase the oxidative stress that is frequently elevated in uremic patients. Our findings also support that hypothesis, because CAS was negatively associated with vitamin/carrier complex levels, reinforcing the importance of determining the vitamin/carrier complex rather than the vitamin plasma levels.

In uremic patients, the increase in vitamin/carrier levels could induce a higher mobilization of the intracellular vitamin pool, with subsequent poorer vitamin utilization by target cells, as has been demonstrated in children with cystic fibrosis (8). Supporting our hypothesis of lower bioavailability of vitamins˚A and E in uremic patients owing to high carrier mobilization are the lower concentrations of those vitamins in mucosal and membrane cells (for example, red cells) (1,2,5,6), and the recognized oxidative stress suffered by uremic individuals (3,7). Because abnormalities in cutaneous vitamin˚A receptors have been hypothesized, but not identified (1), we propose that dialysis patients may suffer high vitamin mobilization, with subsequent pool and target-cell depletion.

By multivariate logistic regression analysis, we found that vitamin˚E/carrier complex, age, and serum albumin are associated, as independent factors, with atherosclerosis (CAS). Age, hypoalbuminemia, hypertension, and diabetes are recognized risk factors for increased oxidative stress (12).

Finally, to improve the real vitamin deficiency, it may be advisable to use oral supplements to increase vitamin˚A and E plasma levels and achieve normal vitamin/carrier complex levels. Although many reports exist of hepatotoxicity associated with high doses of vitamin˚A supplement (15), we believe, on the basis of the Peters and Kelly study (8), that, in maintaining a normal range of vitamin/carrier complex levels, hepatic toxicity is improbable. Moreover, if we intend to improve the nutritional status of dialysis patients with a subsequent increase in prealbumin, RBP, and other proteins (given the strong relationship between vitamins and other antioxidant substances), it would be necessary to give vitamin supplements to maintain adequate levels of vitamin/carrier complex. In addition, malnutrition may modulate the reactive oxygen species that mediate cellular damage through an inability to provide adequate defense if endogenous antioxidants are depleted (16,17). It is also important to control vitamin carrier levels, especially plasma lipids, because hyperlipidemia is present in 70% of dialysis patients (1,2). Moreover, hypercholesterolemia *per se* increases oxidative stress (12), providing another argument for maintaining an adequately controlled lipid profile in PD patients.

Conclusions

Our findings in PD patients suggest intracellular vitamin˚A and E deficiencies, in accordance with measurements of vitamin/carrier complex levels. Elevated vitamin mobilization from pool and target cells is indicated, with risk of subsequent inadequate bioavailability. The determination of vitamin/carrier complexes appears to be more important than that of vitamin plasma levels alone. Vitamin supplementation to achieve adequate vitamin/carrier complex levels may be advisable, especially in hyperlipidemic or malnourished patients.

Our results, and the intracellular vitamin˚A and E deficiencies described by others, may change the traditional concept of hypervitaminosis˚A and E status in uremic patients.

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Leptin as a Marker of Nutrition and Cardiovascular Risk in Peritoneal Dialysis Patients

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Anorexia and protein malnutrition, occasionally associated with obesity, are frequently observed in peritoneal dialysis (PD) patients. Both are recognized risk factors for cardiovascular (CV) morbidity and mortality. Leptin is produced by adipocytes and regulates body-fat mass through a satiety central effect. Leptin accumulates in the uremic state. We analyzed the relationship between plasma leptin levels, nutritional status, obesity, CV risk factors, and atherosclerosis in PD patients.

Leptin was determined using a polyclonal antibody [radioimmunoassay: Linco Research, St. Louis, MO, U.S.A.]. The normal range was 1—7.8 ng/mL. We studied 38 PD patients. Mean leptin levels were 59.1 ± 57.5 ng/mL (elevated in 32 patients). Women (n = 21) showed higher leptin levels than did men (80.4 ± 60 ng/mL vs. 32.3 ± 43.3 ng/mL, p < 0.01), in spite of both groups having a similar body mass index (BMI). A statistically significant direct correlation was found between leptin and BMI (r = 0.7, p < 0.01) and triceps skin-fold measurement (r = 0.77, p < 0.01). Leptin levels and renal creatinine clearance (CCr) showed no significant correlation. Independent of BMI, higher leptin levels were associated with parameters considered to be CV risk factors (Framingham study), such as serum triglycerides < 150 mg/dL (n = 29) as compared with > 150 mg/dL (44.2 ± 53.2 ng/mL vs. 80 ± 58.4 ng/mL, p < 0.05), cholesterol < 250 mg/dL (n = 28) as compared with > 250 mg/dL, (50 ± 55.6 mg/dL vs. 84.7 ± 57.7 mg/dL, p < 0.05), uric acid < 7 mg/dL (n = 28) as compared with > 7 mg/dL (47 ± 53.7 mg/dL vs. 93.1 ± 56.6 mg/dL, p < 0.05), and the presence or lack of presence of left ventricular hypertrophy [68.8 ± 60 (n = 30) vs. 29.5 ± 23.7 (n = 5), p < 0.05].

The patients were classified into two groups according to a clinical atherosclerosis score (CAS). Nineteen patients had low CAS scores, and they showed higher plasma leptin values than did the other patients (82.4 ± 65.7 ng/mL vs 35.8 ± 36.6 ng/mL, p < 0.05). Twelve patients with anorexia had lower leptin values than did patients with normal appetite (19.2 ± 15.8 ng/mL vs. 91.3 ± 58.8 ng/mL, p < 0.001). In non obese patients (BMI < 25 and CCr < 3 mL/min, n = 14), leptin had a statistically significant direct linear correlation with markers of nutrition, including albumin (r = 0.63, p < 0.05), transferrin (r = 0.4, p < 0.05), cholesterol (r = 0.65, p < 0.05), and triglycerides (r = 0.6, p < 0.05). Finally, plasma leptin levels were notably increased in the PD population, indicating increased production (possibly by chronic hyperinsulinism), or uremic retention, or both. By multivariate analysis, we confirmed the association between leptin levels and sex, leptin and BMI, and leptin levels > 40 ng/mL and sex and LVH.

All of those features suggest that plasma leptin levels could be considered a marker of CV risk and food intake in non obese PD patients without inflammation.

Key words
Leptin, nutrition, cardiovascular risk factors

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Introduction

Leptin, a hormone produced by adipocytes (1), regulates body-fat mass through a central satiety effect via neuropeptide Y (NPY) (2). The hormone appears to be cleared by the kidneys, and data on plasma levels are now available for chronic renal failure and for hemodialysis and peritoneal dialysis (PD) patients (3).

Some have speculated that leptin accumulation due to renal failure may be involved in the pathogenesis of uremic anorexia and, in consequence, poor nutritional intake (4,5). Protein malnutrition,

sometimes associated with obesity, is often observed in PD patients (4). Inadequate protein intake and high peritoneal glucose absorption have been involved in the genesis of those nutritional deficits (4,5). Protein malnutrition and obesity (5) are both recognized risk factors for cardiovascular (CV) morbidity and mortality. Obesity is a poorly studied problem in PD patients and is usually associated with insulin resistance, dyslipidemia, CV disease, hypertension, left ventricular hypertrophy (LVH), and diabetes mellitus type˚2 (6). Hyperinsulinemia has been implicated as a primary cause of stimulation of obesity, increased sympathetic nerve activity, and hypertension (7). Obesity and hyperinsulinemia are major stimulators of leptin production, which is positively correlated with fat-mass index (8). Visceral fat mass is associated with early CV morbidity and mortality (6).

Recently, leptin action in the regulation of fat distribution has been demonstrated (9). Interestingly, Dunbar *et al* (10) have demonstrated that leptin injection induces hypertension through renal sympathetic stimulation. We evaluate here the relationship between plasma leptin levels, CV risk factors, atherosclerosis, and nutritional status in PD patients.

Patients and methods

Thirty-eight clinically stable PD patients [32 on continuous ambulatory peritoneal dialysis (CAPD) and 6 on automated peritoneal dialysis (APD)] participated in the study. They included 21˚women and 17˚men ranging in age from 26˚to 85˚years. The mean length of time on PD was 34.5˚± 29.2˚months (range: 3˚— 126˚months). Causes of chronic renal failure were chronic glomerulonephritis (9˚cases), pyelonephritis (8˚cases), nephrosclerosis (7˚cases), polycystic kidney disease (5˚cases), systemic disease (3˚cases), and unknown etiology (6˚cases). Candidates taking thyroid hormones or steroids (including estrogens, contraception, or hormone replacement therapy) were excluded from the study. The presence of anorexia and gastrointestinal symptoms during the preceding months˚was analyzed. Anorexia was estimated by an interview that guided the patient through a survey of 3-day food intake. Six patients presented anorexia, and 6˚patients had anorexia with dyspepsia, nausea, or epigastric pain. Isolated dyspepsia was present in 3˚patients, and epigastric pain in 2˚patients. Twenty-one patients were asymptomatic.

We used a trans-thoracic 2-D Doppler echocardiogram (HP˚Sonos˚1500, 2.25-MHz transducer: Hewlett-Packard, Andover, MA, U.S.A.) to determine LVH [defined as an indexed left ventricular mass (ILVM) greater than 134 g/m^2 in men and 110 g/m^2 in women]. Severe LVH was defined as an ILVM greater than 175 g/m^2 in men and 150 g/m^2 in women (Penn convention).

Severe LVH was present in 5˚patients; medium-to-mild LVH was present in 25˚patients; no LVH was present in 5˚patients; and, for 3˚patients, no data were available. Hypertension was defined as blood pressure˚> 135/85˚mmHg (2˚patients), or a need for antihypertensive drugs (28˚patients). Our patients were also classified according to a clinical atherosclerosis score (CAS) (11). For purposes of multivariate analysis, all patients were grouped into two scores: low CAS (CAS-1 and -2) and high CAS (CAS-3 and -4). We also measured certain CV risk parameters (cholesterol, triglycerides, and uric acid). In accord with Framingham and other longitudinal studies, the values considered to be CV risk levels were 250˚mg/dL for cholesterol, 150˚mg/dL for triglycerides, and 7˚mg/dL for uric acid (12,13).

Obesity was considered to be present when body mass index (BMI) [weight (kg)˚/ height (m^2)] was greater than 25. Obesity grade˚I was between 25 and 30; obesity grade˚II, between 30 and 40; and obesity grade˚III, greater than 40 (14). Extracellular volume status was evaluated by physical examination, blood pressure, peritoneal fluid balances and weight daily follow-up, and anthropometrical assessment [triceps skin fold (TSF) and mid-arm muscle circumference (MAMC)].

Leptin measurement

We used a polyclonal antibody raised in rabbits against highly purified recombinant human leptin (radioimmunoassay: Linco Research, St.˚Louis, MO, U.S.A.). At the sample size, the sensitivity limit was 0.5˚ng/mL, and the linearity was 100˚µg/L. Intra- and inter-assay variation was determined on 5˚human serum samples containing varying leptin concentrations, yielding variations of 7.2˚± 0.4˚ng/mL (intra-assay) and 25.6˚± 0.9˚ng/mL (inter-assay). The intra-assay coefficient was 4.8%, and inter-assay coefficient was 3.5%. Leptin normal range among 115˚healthy subjects (61˚men and 54˚women, aged 15˚— 57˚years, BMI 25.4˚3.2) was 1˚— 7.8˚ng/mL.

Statistical analysis

Linear and Spearman regression analysis were performed. The Student *t*-test for paired and non paired data and the nonparametric comparisons test (Mann—Whitney) were also performed. A p°value less than 0.05 was considered statistically significant. Results are expressed as mean˚± standard deviation throughout the paper.

Univariate and multivariate logistic regression analyses were also performed. The dependent variable (leptin level lower and higher than 40˚ng/mL) was analyzed as a dichotomous entry. The independent continuous variables were serum cholesterol, triglycerides, albumin, uric acid, BMI, creatinine clearance (CCr), Kt/V, and age. The other independent variables analyzed were LVH (yes/no), sex (male/female), and CAS (CAS-1 and -2, CAS-3 and -4).

Results

At baseline, our patients showed these results: hemoglobin, 11.7˚± 1.5˚g/dL; lymphocyte count, 1764.2˚± 1124˚cells/mm^3; creatinine, 10.3˚± 2.77˚mg/dL; urea, 154.3˚± 42.6˚mg/dL; total body protein, 6.8˚± 0.52˚g/dL; albumin, 4.04˚± 0.34˚mg/dL; transferrin, 253.4˚± 50˚mg/dL; cholesterol, 206.6˚± 47.8˚mg/dL; triglycerides, 151.2˚± 68.1˚mg/dL; ferritin 352˚± 360.6˚ng/mL; BMI, 27.2˚± 6.4; TSF, 18.6˚± 10.2˚cm; MAMC, 23.47˚± 5.4˚cm; leptin, 59.1˚± 57.5˚ng/mL; normalized protein catabolic rate (nPCR), 1˚± 0.2˚g/kg daily; and weekly urea Kt/V, 2.1˚± 0.37.

Plasma leptin levels were elevated (>˚7.8˚ng/mL) in 32˚patients (84.2%). Values between 7.9˚ng/mL and 100˚ng/mL were seen in 22˚patients; values between 100˚ng/mL and 200˚ng/mL were seen in 8˚patients; and 2˚patients showed levels higher than 201˚ng/mL. Leptin levels were higher in women than in men [80.4˚± 59.6˚ng/mL (n°= 21) vs. 32.3˚± 43.3˚ng/mL (n°= 17), p°< 0.01]. The mean BMI did not differ between those groups [28˚± 8 (21˚women) vs. 26.3˚± 3.8 (17˚men), nonsignificant].

Leptin and renal function

A statistically significant, negative linear correlation was found between CCr and plasma leptin levels when those levels exceeded 13˚ng/mL (n°= 28: r°= —0.37, p°< 0.05). A even stronger correlation was found when plasma leptin levels exceeded 25˚ng/mL (n°= 25: r°= —0.5p°< 0.01). In both cases, we found no relationship between BMI and CCr (r°= —0.2 andr°= —0.29, both nonsignificant) or BMI and length of time on dialysis (r°= —0.19 andr°= —0.28, both nonsignificant). However, a significant linear correlation was found between length of time on dialysis and CCr (r°= —0.41, p°< 0.01).

Leptin and BMI

According to the scale in a World Health Organization report (14), 8˚patients had a below-normal BMI value (lower than 22.5); 7˚patients, a normal BMI (between 22.5 and 25); 14˚patients, grade˚I obesity (BMI between 25 and 30); and 9˚patients, grade˚II obesity (BMI over 30). Overall, a statistically significant direct linear correlation between plasma leptin and BMI appeared (n°= 38: r°= 0.7, p°< 0.01). Obese patients (BMI˚> 25) showed the strongest correlation (r°= 0.72, p°< 0.01). In addition, higher plasma leptin levels were found in that group (79.5˚± 57.1˚ng/mL vs. 27.9˚± 43.6˚ng/mL,p°< 0.01). No difference in CCr was found between those two groups (1.7˚± 2.09˚mL/min vs. 1.06˚± 1.73˚mL/min, nonsignificant), nor was the time on PD significantly different (29.2˚± 21˚months vs. 42.7˚± 37.9˚months, nonsignificant). Furthermore, in all patients, actual dry weight (r°= 0.53, p°< 0.01), serum triglycerides (r°= —0.36p°< 0.05), and weight gained in the last year (n°= 24: 2.4˚± 3˚kg, r°= 0.44, p°< 0.05) showed significant linear correlations with leptin levels. Similarly, leptin and TSF and MAMC had significant direct correlations at r°= 0.77,p°< 0.01, and r°= 0.44,p°< 0.01, respectively.

Leptin and CV risk factors and CAS

Table˚I shows direct relationships between some CV risk markers and plasma leptin levels. Differences in those results (cholesterol and triglycerides) were similar among patients who received lipid-lowering agents, who had a lower CCr (<˚3˚mL/min), and who were in the group that excluded patients with anorexia. Higher plasma leptin levels were found in patients with LVH, a relationship that could not be explained by differences in BMI (Table˚I), CCr (1.49˚± 2.12˚mL/min vs. 1.45˚± 1.1˚mL/min, nonsignificant) or length of time on PD (20.2˚± 13.3˚months vs. 39.5˚± 30.5˚months, nonsignificant).

Similarly, patients with hypertension showed high leptin values (Table˚I). Ten patients were scored as CAS-1; 9 as CAS-2; 11 as CAS-3; and 8 as CAS-4. Therefore, 19 were assigned to the low CAS group,

TABLE I Relationship between plasma leptin levels and cardiovascular risk factors

Risk factors	*Patients* (n)	*Value*	*Leptin (ng/mL)*	p˚*Value*	*BMI*	p˚*Value*
Uric acid (mg/dL)	28	<7	47±53.7	<0.05	27±7.4	NS
	10	>7	93.1±56.6		28.5±2.5	
Cholesterol (mg/dL)	28	<250	50±55.6	<0.05	27.3±6.9	NS
	10	>250	84.7±57.7		29.8±4.5	
Triglycerides (mg/dL)	29	<150	44.2±53.2	<0.05	27.4±7.6	NS
	9	>150	80±58.4		28.5±4.5	
Hypertension (mmHg)	30	Yes	69.4±59.7	<0.01	27.8±6.9	NS
	8	No	20.4±23.7		26.8±4	
LVH	30	Yes	68.8±60	<0.05	28.3±6.7	NS
	5	No	29.5±23.7		26±3.3	
Albumin (g/dL)	15	<4	44.8±51.7	NS	26.8±5.8	NS
	23	>4	48.4±60.3		27.5±6.9	
BMI	25	<25	35.6±38.3	<0.01		
	13	>25	104.3±62.5			

BMI˚= body mass index; NS˚= nonsignificant; LVH˚= left ventricular hypertrophy.

and 19 to the high CAS group. High plasma leptin levels were associated with a high CAS (82.4˚± 65.7˚ng/mL vs. 35.8˚± 36.6˚ng/mL, p˚< 0.05). However, the BMI was also different (29.7˚± 7.6 vs. 24.6˚± 3.8, p˚< 0.05).

By univariate logistic analysis, significant associations were seen between leptin levels and BMI [odds ratio (OR): 1.36; 95% confidence interval (CI): 1.09˚to 1.71; p˚< 0.009], sex (OR: 11.58; 95% CI: 2.43˚to 55.88; p˚< 0.003), serum triglycerides (OR: 1.01; 95% CI: 1.0002˚to 1.025; p˚= 0.05), and albumin (OR: 9.29; 95% CI: 1.004˚to 86.5; p˚= 0.05). By multiple logistic regression analysis, we confirmed the˚association between leptin levels, sex (OR: 80.87; 95% CI: 4.005˚to 1633.06; p˚= 0.007) and BMI [OR: 1.76; 95% CI: 1.19˚to 2.59; p˚= 0.007 (likelihood ratios model: 29.16; p˚< 0.0001)], and leptin levels, sex (OR: 19.17; 95% CI: 3.29˚to 111.64; p˚= 0.002) and LVH [OR: 14.87; 95% CI: 1.17˚to 187.72; p˚= 0.04 (likelihood ratios model: 17.15; p˚< 0.001)].

Leptin and uremic anorexia

Patients with anorexia (n˚= 6) or anorexia with other gastrointestinal symptoms (n˚= 6) showed lower plasma leptin levels than did asymptomatic patients [19.2˚± 15.8˚ng/mL (n˚= 12) vs. 91.3˚± 58.8˚ng/mL (n˚= 21), p˚< 0.001]. Patients with isolated anorexia showed similar differences [15.8˚± 13.4˚ng/mL (n˚=˚6) vs. 91.3˚± 58.8˚ng/mL (n˚= 21), p˚< 0.001]. A lower BMI value was present in patients with anorexia (23.8˚± 3.3) than in asymptomatic patients (29.5˚± 7.4, p˚< 0.01). Patients with anorexia had lower serum albumin levels than did asymptomatic patients (3.76˚± 0.33˚mg/dL vs. 4.13˚± 0.37˚mg/dL, p˚< 0.05).

Leptin and nutritional status

Leptin plasma levels showed significant, positive linear correlations with markers of nutrition in patients with BMI˚< 25 and CCr˚< 3˚mL/min (n˚= 14): albumin (r˚= 0.63, p˚< 0.05), transferrin (r˚= 0.4, p˚< 0.05), cholesterol (r˚= 0.65, p˚< 0.05), and triglycerides (r˚= 0.6, p˚< 0.05). Lymphocyte count, iron, and nPCR did not show linear correlations. Patients with BMI˚< 25 showed no correlations.

Discussion

Leptin is a 16-kDa protein that regulates body weight (1). Several receptors have been isolated from various tissues, including liver, kidney, and brain (2,3). Under experimental conditions, intraventricular leptin injection demonstrated high-affinity binding to receptors in hypothalamic tissue, where it inhibits hunger and subsequent food intake through NPY secretion (3).

Patients on PD show high plasma leptin levels with respect to normal subjects, as has been described for hemodialysis patients. Excess production and accumulation owing to lack of renal excretion have been suggested as possible causes (3).

Among our PD patients, leptin levels higher than 13˚ng/mL were associated with lower residual renal function. The high plasma leptin value may repre-

sent the correction in the uremic state of the normal leptin level (7.8˚ng/mL for normal renal function). Similarly, a leptin value lower than 4˚ng/mL in normal subjects represents anorexia nervosa (15). That level is not expected among uremic patients, even in intense malnutrition conditions, owing to accumulation of leptin in plasma. In consequence, the accepted normal level must be incremented to interpret correctly the relationship between leptin and nutrition parameters.

Other factors, such as peritoneal glucose absorption could contribute, via insulin release [which increments leptin production (8)], to the higher plasma leptin levels found in PD patients. In fact, *ob* gene expression has been described to increase after food ingestion in rats, perhaps through direct insulin action on adipocytes (9). Peritoneal dialysis may be considered a model for continuous glucose intake and chronic hyperinsulinism. That could be the mechanism that predisposes to obesity, hyperleptinemia, insulin resistance, and *de novo* diabetes in PD patients.

Our obese patients showed strongly positive correlations between BMI and plasma leptin levels. Other investigators also reported that relationship in obese non uremic patients, suggesting a central disorder in the leptin receptor or a post receptor signal transduction defect (16). However, such a relationship was not observed in our non obese patients.

Other uremic factors may possibly influence leptin production or release (17). The greater insulin resistance usually associated with obesity might also be involved (16). Obesity is associated with increased morbidity and mortality. Longitudinal studies have shown significant incidence of hypertension, high cholesterol levels, hypertriglyceridemia with proportional BMI increment, and hyperuricemia, all related to atherosclerosis progression. Obesity and LVH also represent, *per se,* a independent coronary risk factor (13). Regardless of BMI, we found higher plasma leptin levels in patients with elevated values of those CV risk factors (Table˚I). A significant direct linear correlation between triglycerides and leptin was also seen. The hydrolysis of triglycerides stored in adipose tissue produces free fatty acids, which can inhibit glucose utilization by peripheral tissues. That situation could add to the insulin resistance status found in uremic patients (6). Left ventricular hypertrophy is another known effect of hypertension that increases morbidity and mortality in obese patients (13). In patients with LVH, we found high plasma leptin levels that could not be explained by BMI values.

Sympathetic hyperactivity and hyperinsulinism are commonly associated with obesity (7). Experimentally, intracerebroventricular leptin administration increased sympathetic lumbar and renal nerve activity, elevating mean arterial pressure (10). That finding establishes for the first time a relationship between leptin and CV modulation. Also supporting that hypothesis is the observation that leptin decreases brain NPY levels (2), and that NPY intracerebral injection lowers blood pressure (18). On the other hand, sympathetic stimulation or catecholamines may inhibit leptin production, thus creating a negative feedback loop. In chronic renal failure and dialysis patients, clear evidence exists of sympathetic hyperactivity (19), which may be a product of uremic leptin accumulation. Hyperleptinemia could induce hypertension, CV changes (7,8), and dyslipidemia via adrenal hyperactivity (7), increasing atherosclerosis risk in uremic patients. Furthermore, leptin has been found to stimulate the proliferation of glomerular endothelial cells of the rat (20). The endothelial and metabolic effects of hyperleptinemic states, such as uremia, should be studied.

Leptin is a marker of white fat tissue, and interesting studies have associated the central fat-mass distribution with high coronary risk in non uremic (9) and uremic patients (17). High visceral fat mass has been also associated with coronary disease (9,14). Recent experimental studies have shown that leptin is implicated in the distribution of visceral or intra-abdominal fat (9). We thus wonder if leptin could be a marker of visceral fat. Importantly, in multifactorial analysis, we found a relationship between hyperleptinemia, sex (female), and LVH. In patients with a high CAS score, we found an association between high BMI and worse atherosclerosis status, which supports the hypothesis that leptin could contribute to peripheral insulin resistance, hypertension (through increase in sympathetic activity), dyslipidemia, and CV disease in obese PD patients.

In non obese patients, plasma leptin levels nonetheless showed significant direct linear correlation with the studied markers of nutrition. In our study group, leptin could therefore be considered

a marker of nutrition and food intake. With respect to patients with anorexia, we found leptin values lower than those exhibited by the rest of the PD population, probably in relation to their low fat mass.

Finally, we suggest that uremic patients with anorexia might have integrity of their hypothalamic leptin receptor. Their increased leptin values might therefore give rise to an upregulation of the hunger—satiety center.

Conclusion

Hyperleptinemia in PD patients may be considered a CV risk marker. In non obese patients without inflammation, leptin may be a marker of food intake.

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Cholinergic modulation of growth hormone responses to growth hormone-releasing hormone in uraemic patients on peritoneal dialysis

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Summary

BACKGROUND Hypothalamic cholinergic neurotransmission plays a major role in the regulation of GH secretion. Pyridostigmine, a cholinesterase inhibitor, is able to decrease hypothalamic somatostatinergic tone and release GH in normal subjects. Blockade of muscarinic receptor with pirenzepine blunts the GH release in several clinical situations. However, little information is available on the role played by central cholinergic pathways in GH regulation in uraemic patients.

OBJECTIVE We aimed to assess GH responses to GHRH after pretreatment with pyridostigmine and pirenzepine in a group of uraemic patients undergoing peritoneal dialysis (PD). GH responses of the patients treated with recombinant human erythropeitin (rhEPO) were compared to patients without treatment.

DESIGN We studied 14 male patients on PD and nine control subjects. All subjects underwent three endocrine test in random order after an overnight fast. Each subject received GHRH (100 μg, i.v. in bolus at 0 minutes). Sixty minutes before the injection of GHRH subjects were given oral placebo, pyridostigmine (120 mg), or pirenzepine (100 mg).

MEASUREMENTS Blood samples for GH were collected at −60, 0, 15, 30, 45, 60 and 90 minutes The hormonal secretory responses were studied by a time-averaged (area under the curves, AUC) and time-independent (peak values) analysis.

RESULTS Baseline GH concentrations were similar in patients and controls. GH responses to placebo plus GHRH were also comparable in patients and controls (peak 26·6 ± 3·8 *vs.* 33·2 ± 4·4 mU/l, AUC 28·2 ± 3·4 *vs.* 27·8 ± 4·6 mU/h/l). Pyridostigmine administration induced a significant potentiation of GH responses to GHRH both in patients (peak 43·2 ± 5·2 mU/l, AUC 47·6 ± 6·0 mU/h/l; $P < 0{\cdot}01$) and in control subjects (peak 79·2 ± 8·6 mU/l, AUC 78·0 ± 9·4 mU/h/l; $P < 0{\cdot}01$). However, the increment in GH peak and AUC was significantly ($P < 0{\cdot}05$) greater in controls in relation to values found in patients. Pirenzepine administration induced an abolishment of GH release after GHRH stimulation both in PD patients (peak 5·4 ± 2·6 mU/l, AUC 6·0 ± 2·4 mU/h/l; $P < 0{\cdot}01$) and in healthy controls (peak 3·8 ± 0·6 mU/l, AUC 4·0 ± 0·4 mU/h/l; $P < 0{\cdot}05$). Responses to pyridostigmine plus GHRH and pirenzepine plus GHRH were similar in patients on chronic therapy with recombinant human erythropeitin and in patients without rhEPO therapy.

CONCLUSION These results suggest that the cholinergic regulation of GH release is preserved in uraemic patients on peritoneal dialysis. The significantly lower increase in GH response to GHRH induced by pyridostigmine suggests that cholinergic stimulatory tone is attenuated in patients in relation to control subjects. Long-term therapy with rhEPO seems not to affect GH responses to cholinergic stimulation or blockade.

The importance of hypothalamic cholinergic pathways in the neuroregulation of growth hormone (GH) secretion in man has been fully established. In normal subjects, central cholinergic stimulation gives rise to an increase in pituitary GH release, whereas cholinergic blockade is followed by a blunting in GH secretion (Dieguez *et al*., 1988). Pyridostigmine is an inhibitor of acetylcholinesterase and, hence, an indirect activator of cholinergic neurotransmission. This drug has been employed to increase cholinergic tone in different clinical settings. It reduces the release of somatostatin at hypothalamic level, thus increasing spontaneous GH secretion (Friend *et al*., 1997; Coiro *et al*., 1998) and potentiating GH responses to GH-releasing hormone (GHRH) in normal subjects (Massara *et al*., 1986a; Peñalva *et al*., 1990b; Kelijman & Frohman, 1991; Arvat *et al*., 1993; Arvat *et al*., 1997). Conversely, administration of cholinergic receptor antagonist drugs reduces spontaneous GH release as well as GH responses to GHRH, sleep, exercise, L-dopa, glucagon, arginine and clonidine (Massara *et al*., 1986a; Delitala *et al*., 1982; Massara *et al*.,

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1984; Valcavi *et al*., 1991). Since atropine (Casanueva *et al*., 1983) and pirenzepine (Delitala *et al*., 1982; Massara *et al*., 1984), but not nicotine, have shown to attenuate basal and stimulated GH release, a muscarinic receptor is thought to be involved.

Patients with chronic renal failure are known to have several alterations in the regulation of GH secretion. Basal GH concentrations have been found to be increased (Ramírez *et al*., 1978), and abnormal responses to several stimuli such as insulin (Rodger *et al*., 1986), L-dopa (Ramírez *et al*., 1978), arginine (Marumoto *et al*., 1979), thyrotrophin-releasing hormone (Ramírez *et al*., 1978) or glucose (Ramírez *et al*., 1978) have been reported. GH responses to direct pituitary stimulation with GHRH have been found to be normal (Cantalamessa *et al*., 1991) or exaggerated (Ramírez *et al*., 1990) in patients on haemodialysis. Derangements in GH neuroregulation in uraemia have been advocated to account for these findings. However, little information is available on the role played by central somatostatinergic tone in patients with end-stage renal disease, and the effects of pharmacological manipulation of cholinergic activity have not been characterized in this clinical setting. In a previous study we found that oral pirenzepine administration was followed by a blunting in GH responses to GHRH in a small number of patients with end-stage renal disease treated by peritoneal dialysis (PD) (Díez *et al*., 1999a). Based on this background, the present study was performed with the aims of establishing the effects of enhancement and diminution of cholinergic tone by means of the oral administration of pyridostigmine and pirenzepine, respectively, on GH responses to its releasing hormone in a group of uraemic patients undergoing PD, and of assessing whether cholinergic modulation is preserved in these patients or, on the contrary, it could be responsible of some of the reported uraemia-associated derangements in GH secretion. A secondary objective has been to compare the responses obtained in patients treated with recombinant human erythropoietin (rhEPO) with those obtained in patients without this treatment.

Patients and methods

Patients

We studied the cholinergic modulation of GH secretion in 14 uraemic male patients on PD (mean age [± SEM] 44·2 ± 3·4 years; mean duration of dialysis, 11·4 ± 2·1 months) and nine healthy volunteers with normal renal function (mean age, 43·1 ± 4·8 years). The study was approved by the local ethical committee, and informed consent was obtained from all participants before testing. Six patients had end-stage renal disease due to chronic glomerulonephritis, four had interstitial nephropathy, two had polycystic kidney disease, one chronic pyelonephritis and a further one had renal failure of unknown aetiology. There were no significant differences between patients and controls with regard to age and body mass index (24·2 ± 0·7 *vs*. 25·9 ± 0·9 kg/m^2). All patients were found to be stable clinically and adequately dialysed (weekly urea Kt/V, 2·04 ± 0·34). None had diabetes mellitus, severe secondary hyperparathyroidism or other endocrine disorder. Five patients were treated with subcutaneously administered rhEPO (mean dose, 88·0 ± 23·6 U/kg/week; mean duration, 11·2 ± 1·4 months), whereas the remaining nine patients had not received rhEPO therapy. Patients on rhEPO therapy exhibited blood haemoglobin concentrations (11·3 ± 0·4 g/dl) similar than those found in patients without rhEPO therapy (11·2 ± 0·5 g/dl).

Study design

In every patient and control subject, three endocrine tests were performed in random order, on 3 separate days, after an overnight fast. Tests began at 0900 hours with the subject recumbent. An indwelling catheter was placed in a forearm vein and kept patent with a slow infusion of 0·9% NaCl. Each subject received GHRH (GHRH-1–29, Geref; Serono, Spain), 100 μg, i.v. in bolus at 0 minutes Blood samples were collected 60 minutes and immediately before the injection of GHRH and then 15, 30, 45, 60 and 90 minutes after the stimulation. At −60 minutes patients and controls received either oral placebo, or pyridostigmine (Mestinon; Hoffmann-La Roche; Basel, Switzerland), 120 mg p.o., or pirenzepine (Gastrozepin; Boehringer Ingelheim, Germany), 100 mg p.o. All uraemic patients were tested with placebo, pyridostigmine and pirenzepine; nine healthy subjects received placebo and pyridostigmine, and pirenzepine test was performed only in eight controls. In all blood samples plasma GH concentration was assessed. Blood haemoglobin concentration and serum concentration of insulin-like growth factor type I (IGF I), free thyroxine and thyrotrophin were also determined at time 0 in one of the experiments.

Hormone assays

Blood samples were centrifuged immediately and the plasma stored at −20 °C until assayed. Human plasma GH concentrations were determined by using an automated immunoenzymatic assay (AIA 1200, Tosoh Corporation, Tokyo, Japan). Maximal intra-assay and interassay coefficients of variation for the GH assay were 5·4 and 3·3%, respectively. The sensitivity of the GH assay was 0·2 mU/l. IGF-I concentrations were determined using commercially available radioimmunoassay kits (Nichols Institute, San Juan Capistrano, CA, USA) after extraction by acid-ethanol precipitation. Maximal intra- and

interassay coefficients of variation were 3·0 and 8·4%, respectively, and the sensitivity of the assay was 13·5 μg/l. Plasma thyrotrophin concentration was also determined by using the Tosoh immunoenzymatic assay. A heterogeneous competitive immunoassay (Immuno 1 System; Miles, Tarrytown, NY, USA) was used to quantify free thyroxine concentrations.

Statistical analysis

Results are expressed as mean ± SEM. The hormonal responses were studied by a time-averaged (area under the curves, AUC) and time-independent (peak values) analysis. Peak hormonal concentration was considered in each test as the maximum level reached by GH, regardless of the time taken to do so. The AUC for GH was calculated between 0 and 90 minutes by a trapezoidal method. The changes in peak and AUC induced by pyridostigmine plus GHRH and pirenzepine plus GHRH in relation to placebo plus GHRH stimulation were considered in absolute value and in per cent values. For each subject the following parameters were calculated: Δpeak GH after P plus GHRH (mU/l), %Δpeak GH after P plus GHRH, ΔAUC GH after P plus GHRH (mU/h/l), and percentage ΔAUC GH after P plus GHRH; where P is pyridostigmine or pirenzepine, and Δ is increment in relation to placebo plus GHRH.

The statistical evaluation of the GH responses after pyridostigmine plus GHRH and pirenzepine plus GHRH in relation to those obtained after placebo plus GHRH within each group of studied subjects (patients and volunteers) was performed by means of the Friedman analysis of variance by ranks, and the Wilcoxon signed-rank test with the Bonferroni correction was used to determine which pairs of data differed when the Friedman test was significant. For comparisons between patients and controls, the Mann–Whitney test was employed. The Kuskal–Wallis analysis of variance by ranks was used to detect differences between three groups (controls, patients on rhEPO therapy and patients without rhEPO therapy), and the Mann–Whitney test with Bonferroni correction was then used to determine which pairs of groups differed when the Kruskal–Wallis test was significant. The differences were considered to be significant at a P value less than 0·05.

Results

Responses to placebo plus GHRH

Baseline GH concentrations were similar in patients and controls (3·2 ± 0·6 *vs.* 2·0 ± 0·2 mU/l). We did not find any significant differences between the two groups of studied subjects in serum concentrations of IGF I (368·1 ± 46·5 *vs.* 230·2 ± 18·3 μg/l), free thyroxine (1·33 ± 0·10 *vs.* 1·22 ± 0·07 ng/dl) or thyrotrophin (1·44 ± 0·25 *vs.* 1·34 ± 0·17 mU/l). Stimulation with placebo plus GHRH in uraemic patients was followed by an increase in GH levels that reached a maximum of 26·6 ± 3·8 mU/l at 30–60 minutes (Figs 1a, 2a). This maximum did not differ from that found in controls (33·2 ± 4·4 mU/l) at 30–45 minutes (Fig. 1b). The AUC of

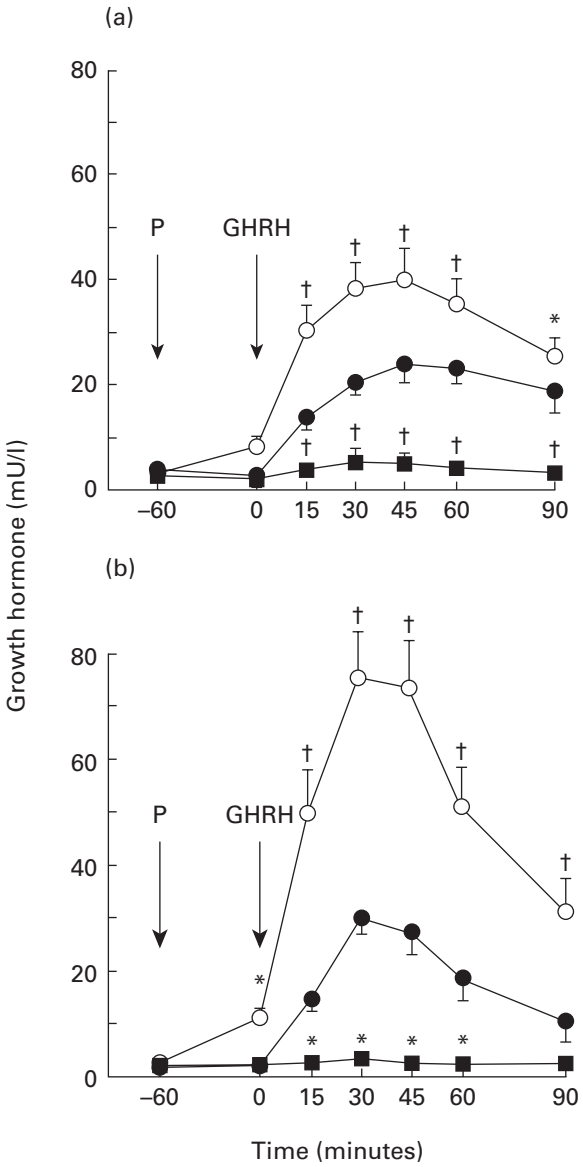

Fig. 1 GH responses to GHRH (100 μg, i.v., arrow) administered 60 minutes after the oral intake (P, arrow) of placebo (●), pyridostigmine (○) or pirenzepine (■) in (a) a group of 14 uraemic patients on peritoneal dialysis and (b) in nine healthy volunteers. Each point represent the mean ± SEM. Responses after pirenzepine plus GHRH were studied in eight controls. *$P < 0{\cdot}016$; †$P < 0{\cdot}01$ (responses after pyridostigmine or pirenzepine *vs.* responses after placebo).

GH was also similar in uraemic patients (28·2 ± 3·4 mU.h/l) and control subjects (27·8 ± 4·6 mU.h/l) (Fig. 2b).

Responses to pyridostigmine plus GHRH

When GHRH was injected 60 minutes after oral administration of pyridostigmine a significant potentiation of GH responses was observed both in uraemic patients (peak 43·2 ± 3·2 mU/l, $P < 0·01$; AUC 47·6 ± 6·0 mU.h/l, $P < 0·01$ *vs.* values after placebo plus GHRH) and healthy volunteers (peak 79·2 ± 8·6, $P < 0·01$; AUC 78·0 ± 9·4 mU.h/l, $P < 0·01$ *vs.* values after placebo plus GHRH). This potentiation reached statistically significant values at 15–90 minutes in patients (Fig. 1A) and at 0–90 minutes in controls (Fig. 1B).

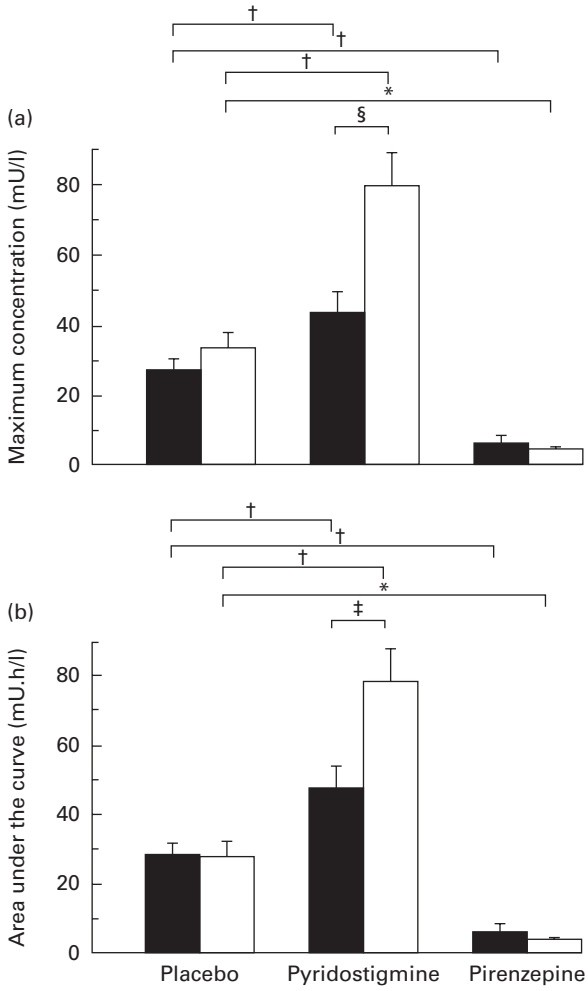

Fig. 2 (a) Maximum GH concentrations and (b) AUC of GH secretion in 14 uraemic patients (■) and nine control subjects (□) in response to GHRH injection 60 minutes after the oral administration of placebo, pyridostigmine and pirenzepine. Each column represents the mean ± SEM. Responses after pirenzepine plus GHRH were studied in eight controls. Comparisons between patients and controls (Mann–Whitney test): ‡$P < 0·05$; §$P < 0·01$. Comparisons within each group (Wilcoxon test with Bonferroni correction): *$P < 0·016$; †$P < 0·01$.

Absolute values for GH peak and GH AUC were significantly higher in healthy volunteers than in uraemic patients ($P < 0·01$ for GH peak, and $P < 0·05$ for GH AUC, Fig. 2). Moreover, the increment in GH peak after pyridostigmine plus GHRH was 16·4 ± 4·0 mU/l (76·1 ± 18·7%) in patients and 46·0 ± 6·0 mU/l (150·5 ± 20·4%, $P < 0·01$) in controls. On the other hand, the increment in GH AUC was 19·4 ± 4·4 mU.h/l (77·8 ± 19·4%) in patients, and 50·2 ± 6·0 mU.h/l (203·9 ± 28·3%, $P < 0·01$) in controls. In summary, increments in GH peak and AUC after pyridostigmine plus GHRH were significantly higher in healthy volunteers with respect to values found in uraemic patients (Fig. 2).

Responses to pirenzepine plus GHRH

Pirenzepine administration induced an abolishment of GH release after GHRH stimulation in both groups of studied subjects (Fig. 1). In patients, a significant reduction was observed both in GH peak (5·4 ± 2·6 mU/l, $P < 0·01$ *vs.* peak after placebo plus GHRH) and in GH AUC (6·0 ± 2·4 mU.h/l, $P < 0·01$ *vs.* AUC after placebo plus GHRH). A similar reduction was found in control subjects (Fig. 2).

The decrement in GH peak and GH AUC were −21·2 ± 2·2 mU/l (− 83·6 ± 3·4%) and −22·2 ± 2·2 mU.h/l (− 75·2 ± 6·9%), respectively, in uraemic patients, and −30·2 ± 4·6 mU/l (− 87·2 ± 2·6%) and −24·4 ± 4·8 mU.h/l (− 83·8 ± 2·4%), respectively, in controls. That is, there were no statistically significant differences between patients and controls in the decrement in GH responses induced by pretreatment with pirenzepine.

Effect of rhEPO therapy on growth hormone responses

We have also studied the maximum concentrations and AUC of GH secretion in the three endocrine tests in control subjects and in patients classified according to the presence or absence of rhEPO therapy. We could not find any significant difference among these groups of subjects in responses after placebo plus GHRH and after pirenzepine plus GHRH. However, Kruskal–Wallis test demonstrated significant differences in peak ($P < 0·05$), Δpeak ($P < 0·01$), AUC ($P < 0·05$), and ΔAUC ($P < 0·01$) after pyridostigmine plus GHRH in the three group of subjects. Nonetheless, when studying differences between pairs of groups (i.e. by using the Mann–Whitney test with Bonferroni correction), we could not find any significant difference between patient treated and not-treated with rhEPO concerning GH responses to pyridostigmine plus GHRH. That

is, although peak, Δpeak, AUC and ΔAUC for GH release after pyridostigmine plus GHRH were higher in patients without rhEPO (peak 48·0 ± 7·4 mU/l; Δpeak 20·8 ± 5·8 mU/l; AUC 53·0 ± 8·8 mU.h/l, ΔAUC 24·8 ± 6·4 mU.h/l) than in patients on chronic rhEPO therapy (peak 34·4 ± 3·6 mU/l; Δpeak 2·6 ± 1·4 mU/l; AUC 38·0 ± 4·0 mU.h/l, ΔAUC 10·0 ± 1·8 mU.h/l), these differences were not statistically significant ($P > 0·05$ in all cases). In other words, we identified a trend to higher responses to pyridostigmine plus GHRH in untreated patients.

Three patients complained of transient dry mouth after pirenzepine. Pyridostigmine caused, borborygmi in one patient, transient abdominal pain in five patients and diarrhoea in three patients. Pyridostigmine and pirenzepine did not cause overt side-effects in any control subject.

Discussion

The group of uraemic patients here studied exhibited normal baseline concentrations of GH and IGF-I, as well as a GHRH-induced GH release similar to that found in controls. Only male patients were selected, as there is sex related difference in the neuroregulation of GH secretion that may be related to altered cholinergic tone (De Marinis *et al*., 1997). We also selected euthyroid, well-nourished and nondiabetic patients in order to avoid variability in GH values derived from conditions such as thyroid dysfunction, malnutrition, obesity or diabetes (Dieguez *et al*., 1988). Our results show that enhancement of cholinergic activity by pyridostigmine induced a striking increment in GH responses to GHRH in both controls and patients. Previous investigations have shown that pyridostigmine potentiates the somatotrope response to GHRH normal subjects (Peñalva *et al*., 1990b), and in different clinical situations with disrupted GH regulation such as acromegaly (Massara *et al*., 1986a), obesity (Scacchi *et al*., 1999), Cushing's disease (Giustina *et al*., 1991), hypothyroidism (Valcavi *et al*., 1993) or insulin-dependent diabetes mellitus (Martina *et al*., 1997). Moreover, pyridostigmine counteracts the inhibitory effect exerted on GH release by exogenous GH infusion (Kelijman & Frohman, 1991), oral glucose (Peñalva *et al*., 1989), intravenous glucose (Delitala *et al*., 1990), oral dexamethasone (Del Balzo *et al*., 1990), free faty acids elevation by lipid-heparin infusion (Peñalva *et al*., 1990a), or repeated GHRH administration (Massara *et al*., 1986b). Experimental studies have shown that the action of pyridostigmine is mediated by a decrease in the hypothalamic release of somatostatin (Locatelli *et al*., 1986), and it has been demonstrated that, in man, pyridostigmine enhances GH secretory burst mass, without altering GH half-life or GH secretory burst duration or frequency (Friend *et al*., 1997).

The observed increment in GH release in PD patients was lower than that found in normal controls. This finding suggests that cholinergic mechanisms of GH regulation are preserved in uraemia, although at a lower level than that present in healthy subjects. In fact, GH peak and GH AUC were increased by 150·5% and 203·9%, respectively, in controls, but only by 76·1% and 77·8%, respectively, in PD patients. There are no previous data on the effect of pyridostigmine on GH secretion in uraemic adult patients. Cappa *et al*. (1991) reported that pyridostigmine increased GHRH-induced GH release in a group of uraemic children with baseline blunted GH responses to GHRH, but failed to potentiate these responses in another group of children with normal GHRH responsiveness. Our data might be accounted for by a decrease in somatostatinergic tone in stable PD patients, and a subsequent lower GH releasing capacity after inhibition of somatostatin secretion. Since pyridostigmine requires endogenous acetylcholine release, our data also seem to indicate that uraemic patients have a reduced central cholinergic tone or a lower acetylcholine releasing capacity than controls.

Results here reported confirm that cholinergic muscarinic blockade with pirenzepine greatly suppresses GH responses in both normal subjects and uraemic patients as previously shown (Díez *et al*., 1999a), suggesting that sensitivity to muscarinic cholinergic receptor blockade is preserved in uraemia. This finding agrees with reports from other investigators who reported that pirenzepine significantly reduced GHRH-induced GH secretion in clinical situations characterized by an altered GH neuroregulation, such as diabetes (Goñi *et al*., 1997), obesity (Maccario *et al*., 1995), anorexia nervosa (Müller & Rolla, 1996), acromegaly (Massara *et al*., 1986a), hyperthyroidism (Valcavi *et al*., 1991) or dementia (Murialdo *et al*., 1990–91). The mechanism by which pirenzepine induces GH suppression is believed to be based on stimulation of endogenous somatostatin release (Casanueva *et al*., 1986). Therefore, our results suggest that GH secretion in uraemia is modulated, at least in part, by acetylcholine-dependent changes in somatostatinergic tone. However, pirenzepine, as well as pyridostigmine, is mainly eliminated by the renal route, and the actions of these drugs might actually be prolonged by their longer elimination half-life in uraemia.

The results also suggest that chronic therapy with rhEPO do not affect GH responses to pharmacological manipulation of cholinergic neurotransmission. We could not find differences between patients treated and not treated with rhEPO in GH responses. We have previously shown that long-term treatment with rhEPO in PD patients potentiates GH responses to GHRH, although this effect did not persist beyond 6 months of therapy (Díez *et al*., 1999b). Interestingly, mean duration of rhEPO therapy in our group of PD patients was 11·2 months, i.e. a time in which no EPO-induced changes in GH response to GHRH were expected according to our previous data.

In conclusion, we have found that male patients on PD exhibited normal GH responses to direct pituitary stimulation

with GHRH. In these patients the increase in cholinergic tone by pyridostigmine induced a clear-cut enhancement in GHRH-induced GH release. Muscarinic blockade with pirenzepine blunted GH responses to its releasing hormone. The potentiation of GH secretion induced by pyridostigmine was found to be lower than that of normal volunteers. These data imply that the cholinergic regulation of GH release is preserved in uraemic patients. The significant lower increase in GH response to GHRH induced by pyridostigmine suggests that cholinergic stimulatory tone is attenuated in patients on PD.

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Disturbances in Appetite Peptide Modulators and Cytokines are Responsible of Eating Behavior Disorders in Peritoneal Dialysis Patients.

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Abstract

Malnutrition is a severe and frequent complication in peritoneal dialysis patients (PD). Eating behavior disorders are deep implicated in the pathogenesis of malnutrition. Anorexia induces a deficit of several nutrients and obesity in PD is frequently associated to protein malnutrition. The present research analyzed the eating behavior disorders in 18 PD patients (twelve with anorexia, twelve obese with high food intake, and eighteen without eating behavior disorders) and a control group with ten heath volunteers. We determined in serum appetite modulator substances at baseline and after a standard food stimuli (Fresubin™, Fresenius, Spain, with 750 Kcal), 30, 60 and 90 minutes. Eating motivation was evaluated a visual analogue scale (VAS) from Hill J et al.

Patients with anorexia showed high satiety before and after to eat, and low desire and pleasure to eat. Neuropeptide Y (NPY) the most potent orexigen known, and cholecystokinin (CCK) a potent anorexigen showed linear correlation with VAS, NPY was associated with more appetite and CCK with lower. Baseline, anorexic patients showed high levels of anorexigen substances, peptide–C, CCK, IL-1, TNF-α and gastric inhibitory peptide gastric inhibitory peptide GIP, and low of NPY. On the contrary, obese group showed higher NPY plasma levels and lower of anorexigens than the remains. Very well defined disorders in the peptide release were found in the different groups.

Patients with anorexia showed a highest and early CCK peak explaining the early satiety sensation measured by VAS. Moreover, NPY stimulation curve was flat with levels decreasing to values lower than baseline at 90 minutes in association with less appetite.

Obese group showed 2 NPY peaks (at 30 and 90 minutes). This explain the great and repeated food intake and the hunger sensation (VAS) showed after 90 minutes from the food stimuli. Insulin and NPY curves were always parallel. IL-1 showed a linear correlation with insulin and GIP, possibly because both are stimulation of insulin secretion. IL-1 and CCK showed also linear correlation and synergic action inducing anorexia. Both IL-1 and TNF-α are pro-inflammatory cytokines that induce insulin resistance and central anorexia. These findings support the inflammatory hypothesis.

Leptin and low nitric oxide (NO_3) values are also anorexigen agents. Leptin participated as anorexigen only at baseline because this not changed with food stimuli. In all studied patients

NO_3 showed a decrease in relation with controls. Abdominal subcutaneous adipose tissue were obtained from elective surgeries and catheter replacement. TNF-α, leptin and adiponectin mRNA expression was measured by real-time RT-PCR. TNF-α was over-expressed in uremic patients, mainly in patients with anorexia. The patron of leptin was very similar, the obese group showed the lowest expression. Adiponectin expression was lower in uremic patients than CG and the obese showed the lowest.

Conclusion, in PD patients, the eating behavior disorders are abnormally modulate by appetite peptides witch are baseline elevated in plasma and are abnormally released. This disorder may be due to renal peptide retention and abnormal inter-relationship between peptide, insulin and cytokines. Uremic carbohydrate intolerance and pro-inflammatory substances appears as novel and important appetite modulator in dialysis patents. Cytokines induces anorexia acting directly in different phases of hunger-satiety cycle.

Introduction

Malnutrition in dialysis patients is definitely associated with high morbidity and mortality (1, 2). This complication is present in near 20-40% of the cases. In peritoneal dialysis (PD) patients, malnutrition is particularly dramatic because peritoneal loss of proteins induces a Kwashiorkor-like syndrome (1).

In PD patients, the lack of appetite is the main obstacle to get an adequate nutritional status (3). Several factors have been described as responsible of uremic anorexia.

One of the most attractive ideas is that in renal disease several endogenous agents modify hunger-satiety cycle by renal retention. These substances normally regulate the appetite as well: cholecystokinin (CCK), leptin, neuropeptide Y (NPY), nitric oxide (NO), gastrin-releasing peptide (GRP) and gastric inhibitory polypeptide (GIP). Overproduction with retention of others molecules that are usually not involved in appetite control might be other explanation, cytokines (tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1)), is the most classic example (3). Both ideas give support to uremic toxin's hypothesis as responsible of uremic anorexia (4). In fact, the hypothesis of anorectic effect by middle molecules isolated from dialysis ultrafiltrate has not been proved (5, 6). The common practice of attributing appetite disorders to insufficient

dialysis dose is not longer sustained. Furthermore, all that endogenous agents are scantly eliminated by dialysis.

Other interesting hypothesis to explain malnutrition and atherosclerosis of dialysis patients as a common pathway, is the inflammatory hypothesis (MIA syndrome or malnutrition, inflammation and atherosclerosis). This suggests that dialysis patients suffer two forms of malnutrition, the type I showed maximal expression of inflammatory mediators with catabolic action, one of this symptom is anorexia (7). However, in the uremic anorexia pathway is only not implicated cytokines as well suggested (3).

The diversity of responses to common situations derived from all these processes is remarkable and has not been clarified. Not all dialysis patients suffer anorexia under similar apparent conditions. This condition points to disorders partially independent of dialysis clearance. Metabolic disorders in the peripheral *via* of hunger-satiety cycle control, have been suggested by our group (8). The peripheral *via* of hunger-satiety cycle control include appetite modulators produced by adipose tissue, stomach, liver and pancreas and the biochemical sing transmitted to brain by blood and vagus nerve.

In the present paper, we hypothesized that disorders in peptide release associated with insulin resistance, inflammatory processes and peptide accumulation by renal insufficiency, induce a variable combination of eating behavior disorders, resulting in anorexia and obesity.

Therefore, with the aim of demonstrate this hypothesis, we performed this study dividing our PD population in three groups based in appetite disorders. We explored the peripheral *via* of hunger-satiety cycle control through the interrelationship among peptide appetite regulators, insulin resistance and inflammatory markers after standardized food intake.

Patients

We included forty-two (20 males and 22 females) clinically stable PD patients. Twenty were on continuous ambulatory peritoneal dialysis (CAPD) and twenty-two in automated peritoneal dialysis (four in CCPD and eighteen on OCPD). The mean age was 56.5 $\pm$ 12.8 years, and the mean period on PD was 35.6 $\pm$ 33.9 months. No acute disorders were present during the two months prior to the study. The causes of chronic renal failure were nephrosclerosis in fifteen, glomerulonephritis in eleven, polycystic kidney disease in eight, unknown in four and systemic disease in four.

Five patients had been diagnosed of hiatus hernia and two of acid pylori disease. Three referred mild occasional gastrointestinal symptoms (pyrosis in three and dyspepsia in two).

We expressly excluded diabetics, patients suffering neoplasia, chronic or acute infections, liver and rheumatoid diseases.

Fourteen patients were ingesting calcium carbonate, twelve aluminum-based phosphate binders, ten oral vitamin D_3 supplements and six, anti-H_2 inhibitors of stomach acid secretion.

Methods

We determined the following parameters:

1.- Dialysis adequacy: urea-KT/V and normalized equivalent of protein-nitrogen appearance (nPNA) (9).

2.- Nutritional markers:

a) Long-term: plasma creatinine, albumin, cholesterol (colorimetric method, Hitachi 704) and transferrin by immunonephelometric method (Boering Nephelometric-Terminal S.A., Spain), serum iron (Hitachi 911), vitamin B_{12} and folic acid (radioimmunoassay).

b) Anthropometric parameters were measured using the recommendations by Frisancho (10), including tricep skin-fold (TSH), midarm circumference (MAC), and midarm muscle circumference (MAMC (cm) = MAC (cm) –3.14 x TSF (cm)). TSF was determined using a caliper (Holtain LTD. , Cross-well, Crymych, Dyfed SA41 3UF. UK).

c) Body composition was determined through bioelectric impedance (BI) (a multi-frequency, Maltron BF 905, USA). Anthropometric data, gender, weight and height were considered. Four electrodes were allocated, two in the non-dominant hand and two in feet, with a separation of 5 cm between electrodes. The decrease in the voltage after the administration of one micro-ampere of electricity, give the impedance or resistance. The software calculates intra and extra cellular body water (liters and %), fat and water-free fat mass (indirect measured of mass muscle), in kg and percentage

d) Medium-term nutritional markers: short half-life proteins, plasma prealbumin, retinal-binding protein (RBP) and anti-thrombin-III (immunonephelometric method). Serum growth hormone (GH) was determined by immunoenzymatic assay (AIA 1200; Tosoh Corporation, Tokio, Japan). Maximal intra- and interassay coefficients of variation were 5.4% and 3.3%, respectively. The sensitivity of GH assay was 0.1 ng/ml (Normal < 5 ng/ml). Serum IGF-I was determined by radioimmunoassay after acid-ethanol extraction (Nichols Institute Diagnostics,

San Juan Capastrano, CA). Maximal intra- and inter-assay coefficients of variation were 2.9 % and 11.4 %, respectively. The sensitivity was 12.9 ng/ml. Normal range was 83-450 ng/ml for younger than 40 years and 54-389 ng/ml for older than 40 years.

e) Short-term nutritional markers: urea nitrogen, serum phosphate and potassium. Mean daily dietary intake was determined from individual 24-hour food records during a 3-day period. Daily calories, carbohydrate, lipid, and protein intake were calculated for each patient using a commercially available computer software (Wander, Sandoz Nutrición, 1990, Barcelona, Spain).

3.- Eating motivation was evaluated through eating motivation scale (visual analogue scale or VAS) by Hill & Blundell (11). VAS included five questions that should be answered before and after eating: desire, hunger and fullness filling, prospective consumption and palatability. The results were giving in a horizontal scale (0-100).

The evaluation of appetite peptide modulator included basaline samples (fasting condition), 30, 60 and 90 minutes after the ingestion of a standard supplement (Fresubin™, Fresenius, Medical Care. Germany) with 750 cc, 750 kcal, 17 g of carbohydrates, 7.5 g of proteins, 5.8 g of fat and 79 ml of water.

Anorexia was defined by a low eating motivation (personal interview and VAS), low food intake (nPNA <1 g/kg/day, daily dietary assessment <30 kcal/kg/day) and low nutritional markers (DOQI clinical practice guideline of nutrition) (12).

Obesity with high food intake was considered when BMI was higher than 30 kg/m^2 (grade I), 30-40 kg/m^2 (II) and > 40 kg/m^2 (III) (WHO criteria) (13), high eating motivation (VAS), high daily food intake or bulimia criteria (DSM-IV) (14).

Normal eating behavior was considered in absence of anorexia and bulimia, normal BMI (18.5-25 kg/m^2) and normal nutritional markers (12).

According to these parameters our patients were divided in three groups: those suffering anorexia (n=5), obesity (n=6) with high food intake and those without eating behavior disorders (n=7). Finally, we include a control group with seven health volunteers.

4.- Plasma or serum peptide appetite modulator were determined by ELISA (enzyme-amplified sensitive immunoassay).

a.- Glucose by hexoquinase reaction (Boehringer Mannhein, Alemania). Fasting normal range 90 y 120 mg/dl.

b.- Insulin (Sorin; Biomedica, Saluggia, Italy). The intra and interassay coefficients of variation were 6.6 % and 6.2 %, respectively. The sensitivity of insulin assay was 3 mIU/ml.

c.- Glucagon (ICN Biomedicals, California, USA). The sensitivity <10 pmol/l, without cross-reaction with enteroglucagon. Normal values 70-90 mg/dL.

d.- C-peptide (Medigenix; Diagnostics Fleurus, Belgium). Maximal intra- and interassay were 7.6 % and 8.8 %, respectively. The sensitivity was 0.1 ng/ml and the normal range was 0.5 to 3 ng/ml.

e.- Neuropeptide Y (NPY) (Peninsula Laboratories, Inc. Belmont, CA, USA). Radioassay. The IC_{50} was 23 pg/100μl (normal range 220-370 pg/ml).

f.- Cholecystokinin (CCK) the 26-33 unsulfated fragment was determined (Peninsula Laboratories, Inc. Belmont, CA, USA). The IC_{50} was 35 pg/100μl (normal values 12-20 pg/ml).

g.- Leptin (Linco Research, St. Louis, MO, USA). The sensitivity was 0.5 ng/ml and linearity of 100 μg/l. The normal range in our laboratory is 3-7.8 ng/ml.

h.- Gastric inhibitory Peptide (GIP) (Peninsula Laboratories, Inc. Belmont, CA, USA). The IC_{50} was 92.88 pg/tube (normal range 35-52 pg/ml).

i.- Ghrelin (RIA, ^{125}-Ighrelin, Linco, research, Inc, Missouri USA), Sensibility 100 pg/ml. The normal range: 900-2500 pg/ml.

j.- Nitric oxide. We measured serum nitrate concentrations (NO_3), a final metabolite of NO, by capillary electrophoresis. Normal values were considered between 90-110 μmol/l (21).

k.- Cytokines with recognized action on hunger-satiety cycle were studied. Baseline levels of tumor necrosis factor (TNF-α) and interleukin 1 (IL-1) were determined by ELISA (enzyme-amplified sensitive immunoassay, Easia Medigenix Diagnostics S.A. Belgium). Values considered normal were 3-20 pg/ml and <15 pg/ml, respectively.

L.- Ghrelin was determined by RIA, 125-Ighrelin: Linco research; sensitivity, 100 ng/ml, normal range in 28 healthy volunteers from our hospital (900-2500 pg/mL).

Adipose tissue cytokine gene expression, abdominal subcutaneous adipose tissue was obtained from elective surgeries and catheter replacement. The sample were put in warm saline and washed twice with saline to eliminate blood and connective tissue. Samples were snap frozen in liquid nitrogen to -80 ºC. Total RNA was isolated with the RNeasy lipid tissue kit (Qiagen, Valencia, CA). The RNA was quantified by measurement of absorbency at 260-280

nm. Cytokine mRNA expression was measured by real-time RT-PCR. The RT-for-PCR Kit (Clontech, Palo alto, CA). We used β-actin to correct the cytokine values.

Statistical Analysis.

Results are given as mean ± SD and range. Comparisons between groups were performed using a non-parametric test, the Mann-Whitney rank-sum U-test. Spearman regression analysis and "t" student tests were used for paired and non-paired data. A "p" value less than 0.05 was considered statistically significant.

To express the statistical differences on the tables, letters "a" through "i" represent significant differences in horizontal sense and symbols in vertical sense.

Results

Demographic and hematological characteristics at baseline are shown in table I. Anorectic patients (group I) were older and showed lower nPNA, albumin, prealbumin, RBP, IGF-I, albumin, TSF, BSF, AMMC, BMI, daily food intake, lean, fat and water body masses by BI and higher plasma levels of TNF-α and IL-1 than the other groups.

Table II shows the data of visual analogue scale (VAS). Anorectic patients also show differences before and after eating than other patients, indicating a poor appetite state. An opposite eating behavior was present in obese group. Table III shows significant linear correlation, positive and negative respectively, between CCK and NPY plasma levels and VAS. Importantly, this dynamic feature confirms a cause-effect relationship between these two endogenous peptides and appetite in PD patients.

Table IV shows the variations in plasma glucose concentration after Fresubin™ intake. All uremic patients show higher baseline glucose, insulin (Table V), glucagon (Table VI) and C-peptide (table VII) levels than control group. Obese patients (Group II) show also higher values of insulin and C-peptide, throughout the whole curve. Group III exhibited a very similar glucose curve than obese patients. In contrast, Group I showed a flat insulin curve until 90 minutes. Regarding the glucagon curve, no differences were found between groups I and II.

Table VIII and Figure 1, show the changes in CCK curve after Fresubin™ intake. At baseline, group I showed high CCK plasma levels than remaining groups. Moreover, anorectic patients increased 3.8-fold CCK levels at 30 minutes after food stimuli. The increment in group II was clearly lesser and retarded (1.3-fold their baseline value, after 60 minutes of food stimuli)

(19.9 ± 4.1 vs. 27.2 ± 6.2 pg/ml, p<0.05). In contrast, non-anorectic non-obese uremic patients (Group III) showed an intermediate CCK peak (2.2-fold). In healthy controls the peak was 3.4-fold at minute 90.

NPY curve under food intake stimuli (Table IX, Figure 2) showed that anorectic patients had lower baseline levels, although into the normal range. The maximal reached value was at 30 minutes but this was non statistically significant (369 ± 26, vs. 405.4 ± 44.2 pg/ml, NS). Importantly, a significant decrease occurred at minute 90 after the stimuli. Obese showed higher baseline NPY values than the others and showed significant increase at minute 30 (463.5 ± 61.6, vs. 605.5 ± 65.7 pg/ml, p<0.05), maintaining stable at minute 90. Controls showed a NPY peak after 30 minutes and decreased at minute 90. The NPY curve from group III was very similar to controls.

Gastrointestinal inhibitory peptide (GIP) plasma levels were higher in uremic patients than in controls (Table X). After food stimuli, groups I, II and control showed GIP peaks at minute 60, whereas obese patients did not show changes.

The leptin levels curve (Table XI) showed baseline differences between the groups but did not change after food stimuli.

Table XII shows the post-prandrial changes in NO_3 plasma values. PD patients showed higher levels than controls (172.2 ± 42.5 vs 92.1 ± 8 μmol/l, p<0.001). All patients showed an important decrease in NO_3 concentration 30 minutes after eating. At 60 minutes, NO_3 increased in all except in obese where this increase occurred at minute 90. Control group showed a progressive NO_3 increase, starting at minute 60.

PD patients showed significant linear correlation between baseline insulin and glucose (r= 0.74, p<0.01), glucose-30´(0.73, p<0.01), glucose-60´, glucose-90´ (0.71, p<0.01), baseline GIP (0.45, p<0.05), GIP-30´ (0.6, p<0.01), GIP-60´ (0.7, p<0.01) and GIP-90´ (0.58, p<0.05).

Insulin-60´ levels showed linear correlation with C-peptide-60´ (0.74, p<0.01), C-peptide-90´(0.79, p<0.01), leptin-60´ (0.67, p<0.01), leptin-90´ (0.56, p<0.05), NPY-30´ (0.62, p<0.01) and NPY-90´ (0.58, p<0.01).

Insulin-90´ showed linear correlation with C-peptide-90´ (0.7, p<0.01), baseline leptin (0.62, p<0.01) and NPY-90´ (0.59, p<0.01) levels.

Serum Leptin at baseline showed negative linear correlation with CCK (-0.51, $p<0.05$), positive with baseline NPY (0.51, $p<0.05$), BMI (0.6, $p<0.01$), fat mass (by BI) (0.67, $p<0.01$) and baseline NO_3 (0.51, $p<0.05$). Leptin-30´ and 60´ showed also linear correlation with NO_3-60´ (0.51, $p<0.05$ and 0.56, $p<0.01$, respectively).

Baseline NPY showed negative linear correlation with baseline IL-1 (-0.52, $p<0.05$) and TNF-α (-0.51, $p<0.05$). NPY-30´ and 90´ was negatively correlated with baseline IL-1 (-0.64, $p<0.01$ y -0.61, $p<0.01$, respectively). NPY-90´showed positive linear with all points of leptin curve (baseline, 30, 60, 90 minutes), (0.51, $p<0.05$, 0.67, $p<0.01$, 0.67, $p<0.01$, 0.63, $p<0.01$, respectively).

TNF-α and IL-1 showed a positive linear correlation (0.85, $p<0.005$), both with baseline IL-1 and CCK (0.45, $p<0.05$) and IL-1 and GIP (0.46, $p<0.05$).

Table XIII shows the post-prandrial changes in Ghrelin plasma values. CG showed an important decrease in ghrelin values after food intake. Ghrelin recover its baseline values 90 minutes after eat. Dialysis patients showed a fat ghrelin curve. Obese showed a fat curve with a very slow decrease at 90 minutes after food intake.

In regard to adipose tissue cytokine gene expression, TNF-α was over-expressed in uremic patients, mainly in patients with anorexia. The patron of leptin was very similar, the obese group showed the lowest expression. Adiponectin expression was lower in uremic patients than CG and the obese showed the lowest (fig. 1a, 1b, 1c).

Figure 1. represent the gene expression of TNF-α, leptin and adiponectin in abdominal fats samples from PD patients getting during peritoneal catheter replacement.

Discussion

Four important findings arise from this study:

1/ the existence of well-defined eating behavior disorders in dialysis patients,

2/ the presence of appetite-related peptide releasing disorders, both spontaneous and after food-intake,

3/ the close relationship between these peptide disorders and the eating behavior in PD patients and,

4/ the strong relationship between peptide disorders and uremic carbohydrate intolerance.

The characterization of appetite disorders is difficult due to the participation of social, cultural, religious and personal conditions (3). Methodologically, Barkeling et al. (11) used a visual analogue scale (VAS) developed by Hill J (15), to measure the eating motivation. Recently, VAS has been successfully used and validated in PD, HD and renal transplanted patients (16, 17). Our results employing this scale demonstrate that all patients show greater fullness before and after lunch and lower palatability sensations. To explain these findings, abdominal discomfort with increase in stomach external pressure by PD fluids and constant peritoneal glucose absorption have been suggested on PD patients (16). The maximal expression of lesser hunger, lower palatability and eating desire, joining with a greater fullness sensation, were found in the anorectic group. Opposite features were found in obese patients (Table II). Curiously, in non-uremic obese patients the eating motivation measured by VAS was not so strongly intense regarding uremics (11). The high plasma levels of NPY (orexigen) and low of CCK (anorexigen agent) (tables I, IV, VII y X), may be responsible of this eating behavior. Other important point is that our patients showed diet preference for carbohydrates, results quite similar to that of Hylander et al (16, 17).

Uremic PD patients confirmed the presence of spontaneous high plasma levels of anorexigen peptides and cytokines, inlcuding glucose, TNF-α, IL-1, insulin, glucagon, CCK, leptin, C-peptide and GIP. These last two with anorexigen effect mediated by insulin. Importantly, anorectic patients showed the highest plasma levels of anorexigen such as TNF-α, IL-1, glucagon, C-peptide and CCK, with the lowest NPY levels (Tables I and IX). Recently, we have found high TNF-α and relatively low NPY plasma levels in a group of PD patients suffering anorexia (18). Experimentally, parenteral or intracerebral administration of TNF-α induces loss of appetite (19, 20). Clinically, TNF-α has been associated with nervous and cancer anorexia, wasting syndrome and rheumatic cachexia (20-23). In uremics, the source of TNF-α is over-production and renal retention. Moreover, the current dialysis technique is not able to eliminate cytokine and, even HD membranes stimulate TNF-α production. Also, peritoneal membrane may result irritated by plastics and PD fluids (24, 25). Inflammed organs are important sources of TNF-α. We recently have shown indirect evidence of excessive TNF-α production in uremic patients with acid peptic disease (18). In other study, the eradication treatment for *Helicobacter pylori* improved serum albumin and anorexia (26). TNF-α has also been associated to uremic

anemia, acidosis, hypertriglyceridemia, uremic neuropathy and cardiopathy (18, 27). As a consequence, we have proposed that TNF-α should be included in the list of uremic toxins (27).

IL-1 is other cytokine associated with many inflammatory diseases (28). Experimentally, the administration of rIL-1 to animals induces loss of appetite (29). Similar to TNF-α, IL-1 exhibits uremic retention (9). According with our results, patients with anorexia showed the lowest residual renal function (RRF) and the highest cytokine concentration (table I). This invites to speculate about the relevance of RRF as more important determinant of appetite than urea-KT/V. These results give also support to the recently postulated hypothesis about the inflammation as cause of malnutrition and atherosclerosis in dialysis patients (30).

Several data support the association between cytokine excess, GI peptides and carbohydrate metabolism abnormalities. High levels of serum cytokines induce peripheral insulin resistance (31, 32). Many GI peptides are stimulated or inhibited by cytokines or insulin. Examples for this, is the synergic action of CCK and IL-1 inducing anorexia (33), and the regulation of GIP, leptin, and NPY by insulin (34-36). In addition, acidosis induced by TNF-α is a diabetogenic factor as well (3, 18). Finally, leptin, a powerful anorexigen and pro-inflammatory molecule, also regulates the appetite and induces insulin resistance. All these relatioships point to the role of insulin resistance in the regulation of hunger-satiety cycle. Our results, showing the paralellism between high insulin, cytokine and peptide plasma levels, agree with the dependence between carbohydrate metabolism and hunger-satiety regulation in PD patients.

In summary, the link between cytokine and peptide appetite modulators could be dependent on the insulin resistance in muscle and fat.

On the dynamic stimulation by food intake

One of the most important findings of this study is the well defined disorder in peptide release after food intake stimuli in the different eating behavior disorders in dialysis patients. In PD patients with anorexia, the plasma levels of CCK were frankly elevated. CCK is a potent anorexigen with peripheral and central actions (37, 38), implicated in the pathogenesis of anorexia nervosa (39), cancer (40), senile (41) and alcoholic (42). CCK is also retained in dialysis patients and not modified by PD or HD (43, 44). However, in a previous study, we have not found high CCK plasma levels in anorectic patients prior to the food stimuli (18). As it is shown in Table VIII and Figure 1, a “peak” of plasma CCK, 30 minutes after eating appeared. This “peak” may be responsible of early fullness sensation (table II and VIII). This CCK “peak”

has also been found in patients with anorexia nervosa (45). Although, in this disorder the exact cause is unknown, the phenomenon is potentially reversible when the nutritional status is recovered (45, 46). This points to some reversible disorder of insulin peripheral action. On the contrary, our obese patients showed a delayed CCK "peak" (table VIII). Again, this abnormality may be found in non-uremic patients with bulimia nervosa, where the CCK "peak" appears later and 50% lower than controls (47),

Importantly, we found linear correlation between CCK and IL-1, IL-1 and GIP, and GIP and insulin. Recently, a synergic effect between IL-1 and CCK inducing anorexia, has been described (33). IL-1 and GIP are stimulating factors to release insulin by the pancreas (34). Therefore, one may speculate that elevated levels of these substances might induce the pseudo-diabetes in uremics. Effectively, in patients with type II diabetes or suffering chronic pancreatitis, GIP are important regulators of insulin secretion. In both cases, high plasma levels are found (35, 48).

Regarding to NPY (table IX, figure 2), patients with anorexia showed prior intake plasma lower levels than the remaining, except the control group. In normal conditions the "peak" of NPY appears 15-30 minutes after the food stimuli. Our results in control group are similar to that of other authors in non-uremic population (49) and we believe that this "peak" is responsible of hunger persistence and increase of gastric emptiness sensation. This explains the popular knowledge that small snakes (tapas) before the main mail are appetite stimulators. Importantly, patients with anorexia did not show this NPY-peak. On the contrary, NPY showed significant decrease at 90 minutes to values lower than baseline, explaining the early and late lack of appetite measured by VAS (table II and III). Obese group showed NPY-peaks at 30 and 90 minutes after food stimuli that could explain the early, late and great quantity of food ingested. In bulimic patients this post-prandrial rebound has been previously described (50). Obese showed NPY levels into the normal range, although higher relative to non-obese. In non-uremic obese population, recent studies suggest disorders in hunger-hypothalamic receptor sensibility (49, 50). In Zuker (*Ob/Ob*) rats, NPY and leptin levels are significantly increased, inducing repeated and compulsive food intakes, due to the lack of inhibition of NPY release by leptin (51). In uremic status, both hypothalamic leptin-receptor and metabolic disorders similar to Zuker rats have not been discarded yet. In other words, since insulin is the main stimulating factor for NPY

release (see parallel curves of insulin and NPY in tables V and IX), and obese showed the highest plasma insulin levels, that would be the reason for high NPY plasma levels found.

But not only the carbohydrate intolerance appears as a regulator of hunger-satiety disorders in uremia. Recently, we have published an inverse relationship between TNF-α and NPY in PD patients (18). We have speculated that TNF-α is an inhibitor for NPY in uremia. Recently, Xu *et al*, (52) have shown the association between a cytokine called "hypothalamic ciliary neurotropic factor" (CNF) and NPY. CNF has a very similar chemical structure to TNF-α and has anorexigen properties mediated by hypothalamic inhibition of NPY release. In a similar sense, and representing inflammation status, we have found a negative correlation between NPY and IL-1 supporting the MIA hypothesis (7).

Leptin is a hormone released by adipose tissue which is retained in dialysis patients and has been associated with malnutrition, via induction of anorexia by decreasing hypothalamic NPY (53, 54). In addition, leptin modulates insulin activity in target cells inducing insulin resistance (55, 56). Therefore, we speculate that baseline high plasma leptin levels found in uremics (both normal and control group) could be another responsible of uremic carbohydrate intolerance. However, in our study plasma leptin did not change 90 minutes after food intake, pointing that leptin-diabetogenic effect can be modulated by the food intake itself. In fact, other authors have published that in normal population plasma leptin increases after 4 hours of food intake (56). We have no data at this term.

Nitric oxide (NO) is another important appetite stimulator (3, 8). Decrease in NO production induces appetite inhibition (57). Moncada's group have described a potent NO inhibitor which accumulates in uremic patients (58). In consequence, one might expect that dialysis patients showed lower NO_3 plasma values than controls. However, we did not found significant differences in these levels. This apparent contradiction may be explained by the uremic retention of inactive forms of NO, represented by NO_3 (59). Anyway, NO_3 is a good marker of NO production in clinic practice (60). Both groups of patients in dialysis showed a decrease in NO production after food stimuli. When we compared obesity and anorexia conditions, the intergroup differences did not reach statistical significance, being all NO_3 values lower than controls. Possibly, NO maintains transcendental and more important relationship with other factors of endothelial origin (58, 60). The relationship between NO and insulin metabolism is determined by the effects of NO on insulin release by pancreas (3, 61, 62) and

the equilibrium with endothelin-1 released by endothelium which induces insulin resistance (61-63). High endothelin-1 plasma levels has been reported in uremics (63). In consequence, the decrease in NO plasma levels 30 minutes after food stimuli that we found, might worse the uremic carbohydrate intolerance. These data remarks the role of insulin on hunger-satiety control.

In regard to fat gene expression, we found an over-expression of TNF-α in anorectic patients, representing a cytokine over-production which is released to the blood inducing systemic effects. Adiponectin showed a down expression which has been described in obeses and severe atherosclerotics.

Finally, table III shows different interesting relationship between eating behavior and peptide appetite modulators. These results are important because demonstrate the importance of using eating behavior surveys and appetite peptide modulators measurements in daily clinical practice. Importantly, several of these peptides participate in mental and physiologic sensations more than gastric fullness (3, 36). According with this idea, NPY increases the desire and the quantity of prospective food intake before eating, but does not participate in the post-prandrial sensation like CCK. Furthermore, CCK does not participate in fullness sensation after eating (table III).

Conclusion. In PD patients, eating behavior disorders are modulated by appetite peptides with peripheral and central action, that are elevated in plasma and abnormally released at baseline conditions. This disorder may be due to renal peptide retention, alterations in their inter-relationship, carbohydrate intolerance and presence of excessive pro-inflammatory cytokines. Cytokines are able to alter the regulation of appetite through direct and indirect mechanisms on the different phases of hunger-satiety cycle.

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Table I. Baseline Differences Between Groups

Parameter	Anoretic (PD) patients	Obeses (PD) pateints	Asymptomatic (PD) pateints	Controls	P
Age (years)	66.4± 10(a,d)	56.3 ± 7.1(b)	49.7 ± 14(a,c)	33 ± 3.7(b,d)	(a,b,c)<0.05, (d)<0.001
DP duration (m)	36.8 ± 32.3	23 ± 11.5	45.5 ± 46.7		NS
CCr (ml/min)	0.5 ± 0.45(a)	1.42± 1.01(b)	1.38 ± 1.39(c)	101 ± 7(a,b,c)	(a,b,c)<0.001
nPNA (g/kg/día)	0.87± 0.21(a)	1.1 ± 0.25	1.14± 0.11(a)		(a)<0.05
KT/V de urea	2 ± 0.25	1.98 ± 0.33	2.17 ± 0.33		NS
Serum Urea (mg/dl)	152 ± 21(a)	159 ± 45(b)	146 ± 51(c)	28.6± 4(a,b,c)	(a,b,c)<0.001
Cr (mg/dl)	10.4 ± 2(a)	11.3 ± 2(b)	10.5 ± 3(c)	1 ± 0.7(a,b,c)	(a,b,c)<0.001
Cholesterol (mg/dl)	174 ± 57.4	211 ± 55.6	188 ± 56	184 ± 30	NS
Albumin (g/dl)	3.7±0.08(a,b)	4 ± 0.2(a)	3.9 ± 0.4	5 ± 0.4(b)	(a,b)<0.05
Transferrin (mg/dl)	209 ± 36	262 ± 47	205 ± 50.7	303 ± 57.2	NS
Prealbumin (mg/dl)	26 ± 7(a,b)	31 ± 2.9(a)	31 ± 7.5	34 ± 3(b)	(a,b)<0.05
RBP (mg/dl)	8.4 ± 3(a,b)	11.5 ± 3(a)	13 ± 2(b)	5.3 ± 1.2	(a,b)<0.05
Lymph. cc/mm3	1298 ± 444	1452 ± 613	1727 ± 480	1877 ± 592	NS
Vitamin B_{12} (pg/ml)	779.3 ± 372.8	973 ± 323.7	893 ± 207.6	516.5 ± 181.6	NS
Folic acid (ng/ml)	5.4 ± 1.5	4.5 ± 1.2	5.5 ± 1.9	7 ± 4.8	NS
Iron (μg/dl)	72 ± 26.7	70.2 ± 16.6	65.8 ± 27	81 ± 27	NS
IGF-I (ng/ml)	257.5± 122(a)	370± 142.7(a)	365 ± 224.6	205 ± 91.2	(a)<0.05
GH (ng/ml)	3.4 ± 3.8	4 ± 4.8	2.2 ± 1.4	1.7 ± 1.7	NS
TSF (cm)	9.5 ± 4(a,b)	24.2± 11.4(a)	22.3 ± 9.4(b)	19.9 ± 10.1	(a,b)<0.05
BSF (cm)	4 ± 0.6(a,b,c)	18.6 ± 7.3(a)	11.4 ± 8.6(b)	13.7 ± 9.5(c)	(a,b,c)<0.05
AMMC (cm)	24.2±1.4(a,b)	27.4 ± 2(a,c)	23.9 ± 13(c)	36.8 ± 13(b)	(a,b,c)<0.05
Diet Survey (kcal/d)	1277 ± 467.4 (a,b,c)	2320 ± 179.4 (a)	2006 ± 351 (b)	2089 ± 339 (c)	(a)<0.01 (b,c)<0.05
Fat (kcal/d)	60.4 ± 28.9 (a,b)	102 ± 23.2 (a,c)	98 ± 22 (b)	74.7 ± 15 (c)	(a,b,c)<0.05
Proteins (kcal/d)	63 ± 18(a)	85.7± 16.6(a)	83.8 ± 13.7	74.5 ± 21.8	(a)<0.05
Carbohydra (kcal/d)	98 ± 41(a,b,c)	227 ± 71(a)	155.5 ± 27(b)	248.8±67.2(c)	(a,b,c)<0.01
BIP. BMI (kg/m^2)	23 ± 2(a)	31.1 ± 3(a,b)	25 ± 2	24 ± 1.5(b)	(a,b)<0.05
BIP. Lean (kg)	22.8±3.7(a,b	30.4 ± 4.5(a)	25.4 ± 2.8	28.6 ± 5.7(b)	(a,b)<0.05
BIP. Fat (kg)	18 ± 5.4(a)	25 ± 8.8(a,b)	13.5 ± 5.3(b)	18.7 ±4.2	(a,b)<0.05
IPB. Water (l)	35.7 ± 5.3(a)	43.7 ± 7.7(a)	37.7 ± 5.4	36.6 ± 5.8	(a,b)<0.05
TNF-α (pg/ml)	121±43.8(a,b)	40 ± 11.6(a)	38.2 ± 16(b)	18 ± 4	(a, b)<0.01
IL-1 (pg/ml)	6.12±0.8(a,b)	2.1 ± 0.43(a)	2.2 ± 1.34(b)	1 ± 0.8	(a,b)<0.001

CCr: creatinin clereance. Cr: serum creatinin. RBP: retinol protein binding. IGF-I: Insulin growth factor type I. GH: growth hormone. TSF: trycipital skin fold. BSF: bicipital skin fold. AMMC: arm muscular mean circunference. BIP: bioelectric impidance. d: day. BMI: body mass index.

Table II. Eating Motivation Measured Through Visual Analogue Scale (VAS) in Peritoneal Dialysis Patients Suffering Eating Behavior Disorders.

VAS	**Patients**	Anorectic (n=12)	Obese (n=12)	Asymptomatic (n=18)	Controls (n=10)	P
Diet survey (kcal/day)		1277 ± 467.4 (a,b,c)	2320 ± 179.4 (a)	2006 ± 351 (b)	2089 ± 339 (c)	(a)<0.01 (b,c)<0.05
Proteins (kcal/day)		63 ± 18(d)	85.7± 16.6(d)	83.8 ± 13.7	74.5 ± 21.8	(d)<0.05
Desire to eat before lunch		60 ± 6.1 (e,f)	76.6 ± 6 (e)	67.8 ± 6.9	72.8 ± 3.9 (f)	(e,f)<0.01
Desire to eat after lunch		8.6 ± 2.2 (g)	21.6 ± 4 (g)	13.2 ± 5	13.5 ± 8.5	(g)<0.05
Hunger before lunch		60 ± 6.1 (h,i,j)	78.3 ± 6 (h)	68.6 ± 4.7 (i)	74.3 ± 4.5 (c,j)	(h)<0.001 (i,j)<0.01
Hunger after lunch		8 ± 4.4 (k,l)	21.6 ± 4 (k)	12.8 ± 5.5	17.1 ± 4.8 (l)	(k,l)<0.01
Fullness before lunch		28 ± 8.4 (m,n)	18.8 ± 2.5	12.5 ± 4.2 (m)	11.8 ± 4.1 (n)	(m,n)<0.01
Fullness after lunch		81 ± 5.4 (o)	59.1 ± 19.6 (o,p)	77 ± 5.6	77 ± 5.6 (p)	(o,p)<0.05
Prospective consumption before lunch		59 ± 5.5 (q,r)	78.3 ± 4 (s)	71.4 ± 3.7 (q,s)	75.7 ± 4.5 (r)	(q,r) <0.001 (s)<0.01
Prospective consumption after lunch		6 ± 2.2 (t,u,v)	25 ± 5.4 (t,w)	12.3 ± 2.7 (u,w)	13.5 ± 4.7 (v)	(t)<0.001 (u,v)<0.01
Palatability		60 ± 7 (x,y,z)	75 ± 5.4 (x)	71.4 ± 4.7 (y)	74.3 ± 5.3 (z)	(x,y,z)<0.01

VAS is measured in a horizontal scale, maxim value 100 mm.

(a-x): statistic differences (read in horizontal).

Table III. Relationship between NPY and CCK Release Response and Visual Analogue Scale

VAS / Peptide	CCK 0	CCK 30	CCK 60	CCK 90	NPY 0	NPY 30	NPY 60	NPY 90
Desire to eat before lunch		-0.6**	-0.52**	-0.4*		0.55**	0.43*	0.55**
Desire to eat after lunch		-0.43*		-0.38*	0.46*	0.54**		0.55**
Hunger before lunch	-0.41*	-0.66**	-0.6**	-0.5*		0.51**		0.45*
Hunger before lunch		-0.55**	-0.52**	-0.53**		0.42*		0.43*
Fullness after lunch					-0.46*	-0.6**		-0.57**
Prospective consumption before lunch		-0.74**	-0.6**	-0.48*	0.48*	0.5**		0.47*
Prospective consumption before lunch		-0.6**	-0.57**	-0.51**		0.8**	0.44*	0.75**
Palatability	-0.5*	-0.63**	-0.75**	-0.5*		0.42*		0.42*
Hunger 2 hous before lunch		-0.74**	-0.6**	-0.6**		0.71**	0.45*	0.63**
Saciety 2 hours after lunch		0.53**	0.42*					

*: p<0.05, **: p<0.01

Note: the results of this table has interest by the capacity of these peptide to modulate the appetite in post-prandrial periodo. Look table VIII (CCK) and IX (NPY) in the diferent groups. These peptides are modulated by insulin secretion, the uremics suffer a diabetes tipe II like fenomenum, therefore the uremic hydrocarbonade intolerance could play a role in the peptide appetite modulator and in consequence induce loss of appetite or obesity.

Table IV. Changes in the Glucose Concentration (mg/dl) After Fresubin Intake.

Time	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	96.5 ± 33 (a,b)	102.5 ± 41	106.7 ± 68 (a)	81.5 ± 4.3 (b)	(a,b) <0.05
Baseline (0 min)	93.6 ± 33 (c,d,β)	101.3 ± 40.5 (e,π,$,#)	105± 69 (d,α,ω,*)	81 ± 5 (c,e,χ,$^{+}$)	(c,d,e) <0.05
30 min	119.4 ± 33 (β)	144.5 ± 45.5 (f,g,π)	131 ± 70 (f,α)	102 ± 17 (g,χ)	(f,g) <0.05
60 min	119 ± 68	156.5 ± 56 (h,$)	156 ± 66.4 (h,ω)	81 ± 14.6 ($^{+}$)	(h) <0.05
90 min	129 ± 76.6	157 ± 80 (i,#)	161.3 ± 82 (*)	75.8 ± 9 (i)	(i) <0.05
post prandial changes (p)	(β)<0.05	(π,$,#) <0.05	(α,ω,*) <0.05	(χ,$^{+}$)<0.05	

Table V. Changes in the Insulin (mIU/ml) After Fresubin Intake.

Time	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	34 ± 23 (a,b)	36 ± 34.6	14.3 ± 6 (a)	12.3 ± 4.8 (b)	(a,b) <0.05
Baseline (0 min)	34 ± 24 (c,d)	41.3 ± 50.7 (e,π)	13.8±4.9 (d,α,ω,*)	12.4 ±3.2 (c,e,χ,$^{+}$)	(c,d,e) <0.05
30 min	55.2 ± 35	135.8 ± 52 (f,g,π)	62.2 ± 48.2 (f,α)	71.4 ± 3.4 (g,χ)	(f,g) <0.05
60 min	80 ± 51.8	158.8 ± 81.7 (h,π)	78.2 ± 43.2 (h,ω)	94 ± 52.8 ($^{+}$)	(h) <0.05
90 min	89 ± 56.5	155 ± 111 (i,π)	79.6 ± 47.1 (*)	58.5 ± 16.8 (i)	(i) <0.05
post prandial changes (p)	NS	(π) <0.05	(α,ω,*) <0.05	(χ,$^{+}$)<0.01	

Table VI. Changes in the Glucagon Concentration (pg/mg) After Fresubin Intake.

Time	Anorectic (PD)	Obesos (PD)	No síntomas (PD)	Controles	(p) entre grupos
Baseline (-15 min)	173.8 ± 77 (a,b)	147.3 ± 41	131.1 ± 32 (a)	76 ± 9.2 (b)	(a,b) <0.05
Baseline (0 min)	170 ± 35.7 (c,d,α)	145 ± 35.9 (π)	130.7 ± 23 (c)	76.4 ± 9.7 (d,χ)	(c,d) <0.05
30 min	181 ± 34 e,α)	168.7 ± 33.3 (f,π)	122.8 ± 29 (g)	75 ± 11 (e,f,g)	(e,f,g) <0.01
60 min	190.6 ± 37.7 (h,β)	160.8 ± 18(i)	133.8 ± 27	83 ± 16.5 (h,i,χ)	(h,i) <0.01
90 min	178.4 ± 30(j,k)	151.5 ± 15	133 ± 29 (k)	80.8 ± 16 (j)	(j) <0.001, (k) <0.05
post prandial changes (p)	(α,β)<0.05	(π) <0.05	NS	(χ)<0.01	

Tabla VII. Changes in the C-peptide Concentration (ng/ml) After Fresubin Intake.

Time	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	18.7 ± 8.6 (a)	17± 6.3 (b)	11.9 ± 4.7 (c)	2.7 ± 0.9 (a,b,c)	(a,b,c) <0.05
Baseline (0 min)	18.2 ± 8 (d)	16.8 ± 6.3 (e,π,$,&)	11.3 ± 4.2 (f,α,ω,*)	2.5±0.6 (d,e,f,χ,$^{+}$)	(d,e,f) <0.05
30 min	19.3 ± 11.2	23.5 ± 7.9 (g,π)	15.9 ± 7 (h,α)	6.9 ± 1.9 (g,h,χ)	(g,h) <0.05
60 min	22.9 ± 14.7	30.7 ± 12.7 (i,$)	21.2 ± 6.4 (j, α,ω)	10.3± 3.5 (i,j, χ,$^{+}$)	(i,j) <0.05
90 min	26.5± 14.6	34.9 ± 14.3 (k,i,&)	23.4 ± 9.7 (l, α,*)	8.4 ± 1.8 (k,l,χ,$^{+}$)	(k,l) <0.05
post prandial changes (p)	NS	(π,$,&) <0.05	(α,ω,*) <0.05	(χ,$^{+}$) <0.01	

Table VIII. Changes in the CCK Concentration (pg/mg) After Fresubin Intake.

Time	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	26.5 ± 3.9 (a,b)	18.7 ± 5.5 (a)	20.7 ± 7.5	11.7 ± 2.9 (b)	(a,b) <0.05
Baseline (0 mim)	25.8 ± 3.7 (c,d,ϕ,∂, Ψ)	19.9 ± 4.1 (c,π)	21.6 ± 8 (d,α,ω,*)	10.9 ± 1.8 (d,e,χ,$^{+}$,λ)	(c,d) <0.05
30 min	95 ± 21.9 (e,f,g,ϕ)	26.5 ± 11.4 (e)	35.1 ± 12.6 (f,α)	29.7 ± 7.8 (g,χ)	(e,g) <0.001 (f) <0.01
60 min	74.8 ± 28 (h,i,j,∂)	27.2 ± 6.2 (h,π)	47.6 ± 14 (i,ω)	33.4 ± 4.5 (j,$^{+}$)	(h,i,j) <0.05
90 min	68.9 ± 32 (k,l,m,Ψ)	20.8 ± 8.4 (k)	35 ± 12.4 (l,*)	37.7 ± 10 (m,λ)	(k,l,m) <0.05
post prandial changes (p)	(ϕ) <0.01, (∂,Ψ) <0.05	(π) <0.05	(α,*) <0.01 (ω) <0.001	(χ,$^{+}$,λ) <0.001	

Table IX. Changes in the NPY Concentration (pg/mg) After Fresubin Intake.

Time	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	367 ± 30.5 (a,b,c)	466.7 ± 76.2 (a)	445.8 ± 60.7 (b)	270.2 ± 29.9 (c)	(a,b,c) <0.05
Baseline (0 min)	369 ± 260 (d,e,f,ϕ)	463.5 ± 61.6 (d,π, λ)	433.7 ± 60.7 (e,α,*)	320.5 ± 47.9 (f,χ,$^{+}$)	(d,e,f) <0.05
30 min	405.4 ± 44.2 (g,h)	605.5 ± 65.7 (g,i,π, β)	492.8 ± 34.5 (h,α)	441 ± 34.3 (g,i,χ, ω)	(g,i) <0.001 (h) <0.01
60 min	358 ± 80	470.7 ± 67 (j,β)	407 ± 84	340.8 ± 52.2 (j, ω,∂)	(j) <0.05
90 min	230.4 ± 73 (k,ϕ)	563 ± 80.7 (k,l,m,π,λ)	377 ± 68.7 (l,*)	256.8 ± 77.4 (i,m,$^{+}$)	(j) <0.001 (k,l,m) <0.01
post prandial changes (p)	(ϕ) <0.01	(π) <0.01, (λ,β) <0.05	(α,*) <0.05	(χ,ω) <0.001 ($^{+}$,∂) <0.05	

Table X. Changes in the GIP Concentration (pg/mg) After Fresubin Intake.

Tiempo	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	106.8 ± 23.5 (a)	143.3 ± 82.6 (b)	131.6 ± 25.3 (c)	50.6 ± 11.6 (a,b,c)	(a,b,c)<0.01
Baseline (0 min)	101.2 ± 22 (d,ϕ,∂)	111.8 ± 27.7 (e,π)	132.6 ± 25 (f,α)	47.1 ± 7.3 (a,e,f,χ,+,ω)	(d,e,f)<0.01
30 min	130.4 ± 38.5 (g)	145.9 ± 29.2 (h,π)	140.5 ± 42.2 (i)	84.7 ± 9.6 (g,h,i,χ)	(g,h,i) <0.01
60 min	142.2 ± 24.1 (j,ϕ)	159.6 ± 44.1 (k,π)	196.3 ± 90.7 (f)	100.8 ± 13 (j,k,+)	(j,k) <0.05
90 min	136.8 ± 33.8 (∂)	188.6 ± 80.4 (i,π)	190.5 ± 68.4 (l,α)	94 ± 6.3 (l,ω)	(l) <0.05
post prandial changes (p)	(ϕ) <0.01, (∂) <0.05	(π) <0.05	(α) <0.05	(χ,ω) <0.05 (+)<0.01	

Table XI. Changes in the Leptin Concentration (ng/ml) After Fresubin Intake.

Time	Anorectic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls
Baseline (-15 min)	43 ± 46	134 ± 66	36.8 ± 29	10.7 ± 7
Baseline (0 min)	44.5±45(a,d)	110 ± 45(a,b,c)	35 ± 28(b,e)	11.8 ± 8(c,d,e)
30 min	39.7 ± 40	125 ± 67	33 ± 26.2	9.6 ± 7.2
60 min	41 ± 42	111 ± 50	31 ± 19.7	11.9 ± 9.3
90 min	37.6 ± 39	117 ± 56.6	29.7 ± 23	12.5 ± 10

p: (a,b,c,d,e)<0.01

Table XII. Changes in the NO_3 Concentration (μmol/l) After Fresubin Intake.

Time	Anoretic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	174.2 ± 60 (a)	190 ± 36 (b)	153.9 ± 25.4 (c)	92.1 ± 8 (a,b,c)	(a,c)<0.001 (b)<0.05
Baseline (0 min)	175 ± 59.9 (d,β)	190 ± 35.9 (e,π,Φ)	152 ± 26.6 (f,α,ω)	92.7 ± 7.5 (d,e,f,χ,$)	(d,f) <0.001 (e)<0.05
30 min	113.4 ± 31.8 (g,β,δ)	109.2 ± 39.5 (Φ)	103.1 ± 31.6 (α)	87.1 ± 6.8 (g)	(g) <0.05
60 min	148.7 ± 21.3 (δ)	159.3 ± 58.4 (#)	80 ± 57 (ω)	125.5 ± 39 (χ)	NS
90 min	155.4 ± 55	106.7 ± 42.6 (π,#)	119.2 ± 95	138.2 ± 52.4 ($)	NS
Post prandial changes (p)	(β)<0.01 (δ)<0.05	(π,Φ)<0.01 (#)<0.05	(α)<0.01 (ω) <0.05	(χ,$)<0.05	

Table XIII. Changes in the Ghrelin Concentration (pg/mL) After Fresubin Intake.

Time	Anoretic (PD)	Obeses (PD)	Asymptomatics (PD)	Controls	(p) between groups
Baseline (-15 min)	174.2 ± 60 (a)	190 ± 36 (b)	153.9 ± 25.4 (c)	92.1 ± 8 (a,b,c)	(a,c)<0.001 (b)<0.05
Baseline (0 min)	175 ± 59.9 (d,β)	190 ± 35.9 (e,π,Φ)	152 ± 26.6 (f,α,ω)	92.7 ± 7.5 (d,e,f,χ,$)	(d,f) <0.001 (e)<0.05
30 min	113.4 ± 31.8 (g,β,δ)	109.2 ± 39.5 (Φ)	103.1 ± 31.6 (α)	87.1 ± 6.8 (g)	(g) <0.05
60 min	148.7 ± 21.3 (δ)	159.3 ± 58.4 (#)	80 ± 57 (ω)	125.5 ± 39 (χ)	NS
90 min	155.4 ± 55	106.7 ± 42.6 (π,#)	119.2 ± 95	138.2 ± 52.4 ($)	NS
Post prandial changes (p)	(β)<0.01 (δ)<0.05	(π,Φ)<0.01 (#)<0.05	(α)<0.01 (ω) <0.05	(χ,$)<0.05	

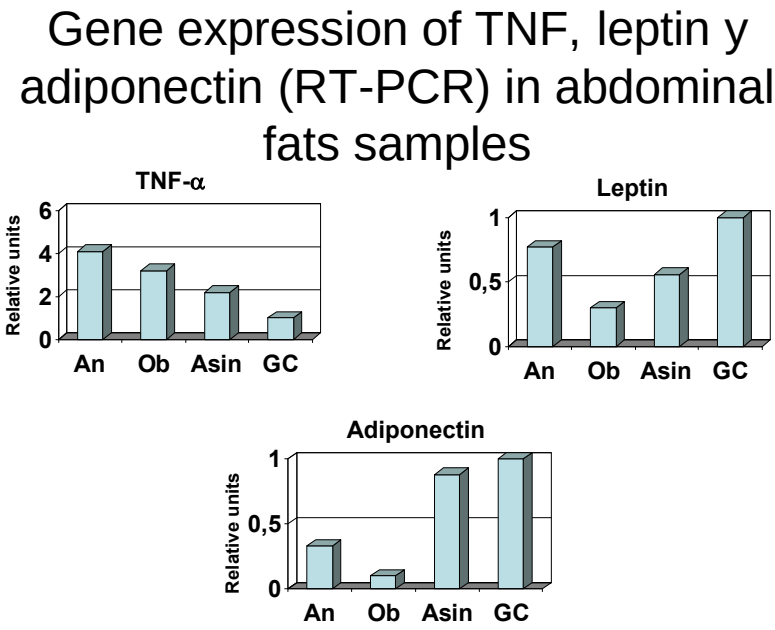

Figure 1.- Gene expression of TNF-α, leptin and adiponectin in abdominal fats samples from PD patients getting during peritoneal catheter replacement.

Treatment of Anorexia and Malnutrition in Peritoneal Dialysis Patients with Megestrol Acetate

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Anorexia and malnutrition are common complications and powerful predictors of morbidity and mortality in peritoneal dialysis (PD) patients. Megestrol acetate (MA) is a progestogen that has been demonstrated to increase appetite and weight in patients with cancer or acquired immunodeficiency syndrome. To determine whether MA might benefit PD patients, we treated 32 patients with 160 mg MA daily.

Treatment lasted a mean of 5.93 ± 5.12 months (range: 1 – 23 months). In 68.8% of the patients, appetite improved. Weight gain was statistically significant starting in the third month (initial weight: 66.5 ± 11.4 kg; weight at third month: 68 ± 10.4 kg; p < *0.05). We observed a nonsignificant increase in serum albumin at the third treatment month (initial serum albumin: 3.44 ± 0.27 g/L; serum albumin at third month: 3.54 ± 0.27 g/L;* p = *0.45). No side effects were observed.*

Our experience suggests that treatment with 160 mg MA daily in PD patients leads to an increase in appetite, serum albumin, and weight gain in most patients, with no negative side effects.

Key words
Megestrol acetate, malnutrition, anorexia

Introduction

Anorexia and malnutrition are common complications and powerful predictors of morbidity and mortality in peritoneal dialysis (PD) patients (1,2). Treatment modifications that can help to prevent or treat malnutrition are adequate dialysis dose, avoidance of acidemia, and addition of food supplements. More experimental forms of nutrition therapy (3,4) include dialytic nutrition, appetite stimulants [for example, megestrol acetate (MA)], and growth factors (anabolic recombinant human growth hormone or insulin-like growth factor I).

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The appetite stimulant MA is a semi-synthetic steroid progestogen that was originally used as therapy in metastatic breast and endometrial cancer. What was originally considered the most frequent side effect of MA treatment—increased appetite and body weight—has gradually become an established treatment for malnutrition in patients with acquired immunodeficiency syndrome or non hormonal–responsive cancer (5,6).

The four published studies that evaluated MA in end-stage renal disease (ESRD) populations (3,7,8,9) had varying results and conclusions. Our purpose in the present study was to (A) determine whether MA increases appetite and improves parameters of nutrition in PD patients and (B) explore the required dose and MA side effects.

Patients and methods

Between January 1995 and March 2001, we retrospectively recruited 163 PD patients from our PD unit. Within that cohort, we treated 32 patients who had anorexia and malnutrition with 160 mg MA daily.

We evaluated whether MA increased appetite and weight, and whether it improved these parameters of nutrition: serum albumin, cholesterol, triglycerides, lymphocyte count, transferrin, and protein catabolic rate (PCR). We also recorded the appearance of any side effects.

Table I shows demographic information, Kt/V, and causes of ESRD for the 32 study patients (19 men, 13 women; 19 on continuous ambulatory PD, 13 on automated PD). Median age of the patients was 64.19 ± 13.8 years. The initial Kt/V for the group was 2.14 ± 0.56. Twenty patients were receiving erythropoietin treatment.

Statistical analysis

Results for normally distributed continuous variables are expressed as mean ± standard deviation, and $p <$

TABLE I Characteristics of the study patients

Patients (*n*)	32
Mean age (years)	64.1±13.8
Sex (M/F)	19/13
Mean dialysis duration (years)	3.93±3.25
Mean weekly Kt/V	2.14±0.56
Time on MA treatment (months)	5.93±5.12
Cause of end-stage renal disease (*n*)	
Diabetes	9
Glomerulonephritis	5
Nephrosclerosis	5
Unknown	5
Interstitial nephritis	3
Systemic lupus erythematosus	2
Polycystic kidney disease	2
Vasculitis	1

M/F = male/female; MA = megestrol acetate.

0.05 was considered statistically significant. Continuous variables were analyzed using the paired Student *t*-test and the Wilcoxon *t*-test as appropriate.

Results

The mean duration of MA treatment was 5.93 ± 5.12 months (range: 1 – 23 months). Appetite increased in 22 of the 32 patients (68.8%). Megestrol acetate was stopped in 27 patients for these reasons: because appetite increased ($n = 10$); because the patients died from causes unrelated to MA therapy ($n = 7$); because appetite failed to increase ($n = 8$); and because the patients underwent renal transplantation ($n = 2$).

Weight gain during the first 2 months was statistically nonsignificant (initial weight: 66.5 ± 11.4 kg; weight at first month: 67.2 ± 11.8 kg; $p = 0.82$), but weight gain became statistically significant starting at the third month (weight at third month: 68 ± 10.4 kg; $p < 0.05$). We observed a nonsignificant increase in serum albumin from the third treatment month (initial serum albumin: 3.44 ± 0.27 g/L; serum albumin at third month: 3.5 ± 0.27 g/L; $p = 0.45$). The patients' PCR increased significantly from the third treatment month (initial PCR: 0.95 ± 0.3 2 g/kg/day; PCR at third month: 1.13 ± 0.45 g/kg/day; $p = 0.032$). Except for cholesterol, the other parameters of nutrition (triglycerides, lymphocyte count, transferrin) increased beginning in the first month; however, the increase did not become statistically significant (Table II). No patients presented with side effects during treatment with 160 mg MA daily.

Discussion

Malnutrition is a common complication associated with high mortality and morbidity in dialysis patients (1,2,8). Malnutrition in dialysis patients has multifactorial causes.

Anorexia is probably the most important cause of inadequate dietary energy and protein intake in long-term dialysis patients (1,3). Loss of appetite may be related to several factors, including drug–nutrient interactions, cytokine effects on the central nervous system, depression, poverty, and loneliness (3,10).

Interventions to treat malnutrition in dialysis patients include both dialytic and nutritional approaches (3). The first step should always be an evaluation of the dialysis dose. Underdialysis has been associated with increased morbidity and mortality in dialysis patients and may induce anorexia and nausea (11). To date, nutritional interventions have encouraged supplementation by the enteral or parenteral route or both (3). A potential new therapy for treating malnutrition in dialysis patients is the use of appetite stimulants, in particular MA (3,5), a semi-synthetic steroid progestogen. It is an orally active derivative of the naturally occurring hormone progesterone.

Oncologists observed that MA, used in the therapy of patients with metastatic breast and endometrial cancers, was associated with appetite stimulation and body weight gain. The exact mechanism by which MA stimulates appetite is still largely unknown. The effect has been postulated to be at least partly mediated by neuropeptide Y, a potent central appetite stimulant that also inhibits the *in vitro* production of cytokines, including tumor necrosis factor alpha, interleukin-1, and interleukin-6 (5). Most of the weight gain came from increased adipose tissue. Karcic *et al.* demonstrated that, *in vitro,* MA is a potent inducer of lipocyte differentiation (5,8).

Several studies on MA as treatment for malnutrition in patients with cancer or acquired immunodeficiency syndrome have been published (12–16). In the study by Loprinzi *et al.* (13), 133 patients with cancer were randomly assigned to receive 800 mg MA daily or a placebo. Patients assigned to MA more frequently reported improved appetite ($p = 0.003$) and food intake ($p = 0.009$) as compared with patients receiving placebo. Von Roenn *et al.* (12) studied 271 cachectic patients with acquired immunodeficiency syndrome, randomly assigning them to receive placebo or 100 mg, 400 mg, or 800 mg MA daily for 12 weeks.

TABLE II Body weight and parameters of nutrition at initiation of megestrol acetate treatment and at 3 months

	Month 0	*Month 3*	p *Value*
Body weight (kg)	66.5±11.4	68±10.4	<0.05
Serum albumin (g/L)	3.44±0.27	3.54±0.27	NS
Protein catabolic rate (g/kg/day)	0.95±0.32	1.13±0.45	<0.05
Cholesterol (mg/dL)	190.8±47.1	187.87±50.4	NS
Triglycerides (mg/dL)	144.1±86.1	147.1±73.1	NS
Lymphocyte count (cells/mL)	1457.92±813.7	1667.3±579.26	NS
Transferrin (mg/dL)	212.81±58.3	223.4±52.04	NS

NS = nonsignificant.

The MA stimulated appetite, food intake, and body weight gain and improved the patients' overall sense of well-being.

However, there are several problems with the information found in the literature about MA. The principal problem is that of appropriate dosing. Most studies used a dose of 800 mg daily (6,12,13). One of the studies also employed a range of doses from as low as 160 mg daily to as high as 1280 mg daily (13). Between those extremes, other studies have used dosing levels of 160 mg daily (13,14), 320 mg daily (14), and 480 mg daily (15). Our study used the low, 160 mg daily dose.

The second problem has to do with the contradictory findings about the toxicity of MA. Some investigators found only limited or no side effects (6,13,15). Others (6,12) reported significant side effects, including encephalopathy, depression, hypervolemia, impotence, irregular menses, rash, fluid retention, and diarrhea. In our study population, no side effects were observed at the 160 mg daily dose.

The third problem is the nature of the measured benefit from MA. Lien and Ruffenach (8) found an increase in serum albumin. Tchekmedyian (16) found no increase in survival. We found a nonsignificant increase in serum albumin and in other parameters of nutrition, except for cholesterol (Table II).

We are aware of four small studies to date that examine the effect of MA in dialysis patients. Lien and Ruffenach (8) studied the effect of a low MA dose (40 mg daily) in 16 malnourished dialysis patients. Serum albumin increased in 75% of the patients, and all of the responders reported an increase in food intake because of improved appetite. However, no significant weight gain occurred, and 1 patient stopped MA because of vaginal bleeding. Williams *et al.* (9) found that a low dose of MA (160 mg daily) was not effective in increasing serum albumin level or lean body mass in a small group of malnourished hemodialysis patients.

Burrowes *et al.* (3) reported on a hemodialysis patient with hypoalbuminemia who received MA for 24 weeks. That patient was started on a moderate dose of MA (320 mg daily) that was increased to 440 mg daily at week 13, and to 560 mg daily at week 20. The patient's serum albumin level was maintained, but fat increased by 163%. The patient did not experience any other side effects.

Boccanfuso *et al.* (7) studied the effect of 400 mg MA twice daily in 17 dialysis patients. Those patients reported improved appetite and showed an increase in dry weight, but no increase in serum albumin. However, reported side effects included diarrhea, confusion, hyperglycemia, headaches, dizziness, and elevated lactate dehydrogenase.

In our study, 32 peritoneal dialysis patients received 160 mg MA daily over a median period of 5.93 ± 5.12 months. Appetite improved in 68.8% of the patients. We observed statistically nonsignificant increases in serum albumin and statistically significant weight gain from the third month. No side effects were observed.

Conclusions

Megestrol acetate is a well-established treatment for anorexia and cachexia associated with cancers and acquired immunodeficiency syndrome. Our experience suggests that, in PD patients, treatment with 160 mg MA daily increases appetite significantly and serum albumin nonsignificantly, and produces significant weight gain in most treated patients without producing side effects. However, a few small, controversial studies of anorexia in dialysis patients treated with MA also exist. Further studies are needed to fully

evaluate the role of MA in the treatment of anorexia and cachexia in dialysis patients.

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Assessing 24-Hour Blood Glucose Patterns in Diabetic Patients Treated by Peritoneal Dialysis

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The minute-to-minute effect on blood glucose levels of high-dextrose peritoneal dialysate is not known. We arranged for 7 patients with diabetes, treated by peritoneal dialysis (PD), to wear a continuous glucose monitoring system (CGMS: Medtronic MiniMed, Northridge, CA, U.S.A.). A sensor was inserted subcutaneously into the skin of the patient's abdomen or back to measure glucose in the interstitial fluid. Readings were recorded every 5 minutes for up to 72 hours. The portion of the day during which the patient's blood glucose levels were greater than 180 mg/dL (calculated as a percentage of time) was recorded. Most of the patients participating in the study had elevated levels of glycohemoglobin and hemoglobin A1c, and, for a large percentage of the day, showed blood glucose tracings well above the recommended standards of control. Representative CGMS tracings from patients with type 1 and type 2 diabetes are shown.

Key words
Continuous blood glucose monitor

Introduction

Good glycemic control is often difficult to obtain in diabetic patients treated by peritoneal dialysis (PD). The minute-to-minute effect on blood glucose levels of peritoneal dialysate containing high concentrations of glucose is not known. Current practice is to teach patients to monitor blood glucose levels at home by applying finger-stick blood to a home glucose monitor. To determine overall control of blood glucose, hemoglobin A1c (HbA1c) values are measured at regular intervals in the clinic.

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However, uremia and treatment with PD may alter the relationships between blood glucose patterns and HbA1c. To understand those interactions, we compared standard measures with the blood glucose pattern obtained using a continuous glucose monitoring system [CGMS: Medtronic MiniMed, Northridge, CA. U.S.A. (1)].

Patients and methods

We arranged for 7 patients with diabetes, treated by PD, to wear a CGMS. A sensor inserted subcutaneously into the skin of the patient's abdomen or back sends signals to a recording device every 5 minutes, yielding approximately 288 readings in 24 hours. To calibrate the CGMS readings, each patient was also asked to perform finger-stick blood-sugar measurements using a traditional blood glucose meter at least 4 times daily. The patients were asked to keep a log of diet, insulin (or oral hypoglycemic medication), exercise, and PD exchanges and treatments. The CGMS monitoring was repeated at 1- to 3-month intervals, after dietary or medical interventions.

Readings by the CGMS were not visible in real time, but only after the sensor was removed, and the data downloaded to a computer. The portion of the day during which the wearer's blood glucose levels exceeded 180 mg/dL (as a percentage of time) was calculated.

Each study was conducted over 3 consecutive days.

- On day 1 the Medtronic MiniMed CGMS was applied to the patient, and the patient was educated in the use of the monitor.
- On day 2 the patient returned for a fasting blood draw in the morning. Blood was drawn to measure HbA1c and glycohemoglobin.
- On day 3 the CGMS was removed, and the results were analyzed and reviewed with the patient.

At the time of the study, patients were being treated with several hypoglycemic regimens, including oral agents or insulin combinations [glargine and lispro, or neutral protamine hagedorn (NPH) and regular insulin]. Results of the CGMS readings were shared with study participants as a tool to attempt to improve glycemic control. In addition, participants received counseling in nutrition and diabetes management from a registered dietician and other medical professionals.

TABLE I Markers of hyperglycemia in the study patients

	Date	*>180 mg/dL (% time)*	*GHb (%)*	*HbA1c (%)*
Patient 1	April 2003	1	7.3	7.5
	May 2003	56	7.4	8.0
	June 2003	51	7.4	8.9
	July 2003	52	9.8	8.3
	September 2003	38		9.0
	November 2003	23		8.5
Patient 2	January 2003	32	9.1	7.9
	March 2003	26	8.1	7.3
Patient 3	February 2003	42	7.5	
	March 2003	21	7.9	7.3
	June 2003	66	7.9	8.1
Patient 4	February 2003	39	9.3	7.9
Patient 5	September 2003	67		7.1
Patient 6	September 2003	43		
	October 2003	48	6.4	7.0
Patient 7	January 2004	11		6.4

GHb = glycohemoglobin; HbA1c = hemoglobin A1c.

Results

Most of the patients participating in the study had elevated levels of glycohemoglobin or HbA1c, or both. Table I summarizes the percentage of time the patients' blood glucose levels exceeded 180 mg/dL, as compared with more standard measures.

A general concordance between measures is noted in this preliminary, observational study. Two representative CGMS tracings are shown (Figures 1 and 2). Meals, exercise, dialysis exchanges, and medication can be seen to have a direct temporal impact on blood glucose values.

Discussion

Blood glucose levels are well above the recommended standards of control for a large portion of the day in diabetic patients treated by PD. In some patients, a clear increase in blood glucose occurs after a peritoneal exchange or in response to initiating cycler exchanges. One important observation is that finger-stick blood glucose determinations are accurate. However, because finger-stick glucose levels are performed intermittently, they may miss many hours of markedly higher or lower readings. In Figure 2, the effect of intraperitoneal insulin is particularly rapid, but a marked rebound occurs after meals or dialysis exchanges. Because the role of PD solution with its soluble glucose is incompletely understood, more study is needed.

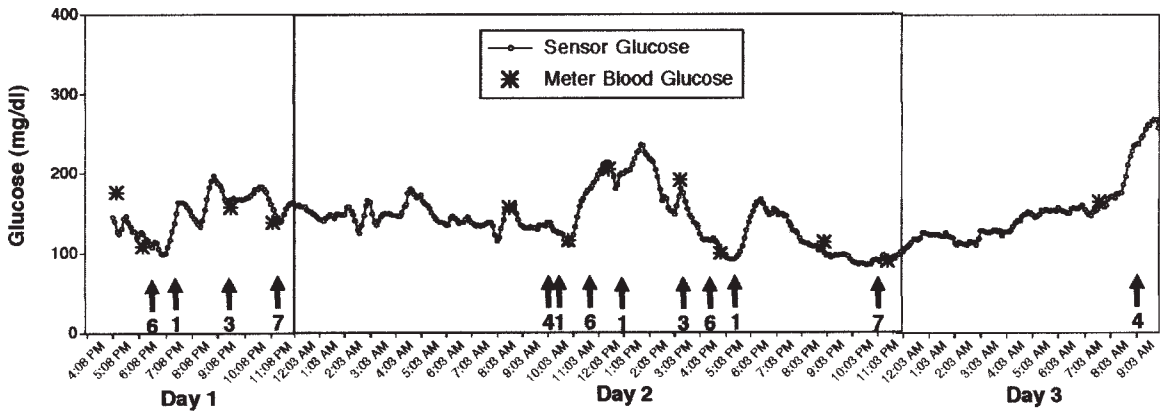

FIGURE 1 Continuous blood glucose monitor (CGMS) pattern in 74-year-old man with type 2 diabetes, treated with oral hypoglycemic agent. 1 = meal; 2 = snack; 3 = exercise; 4 = oral agent; 5 = short-acting insulin; 6 = PD exchange; 7 = initiation of cycler exchanges. Note glycemic response to meals (code number 1), and effect of exercise (code number 3) to lower blood glucose.

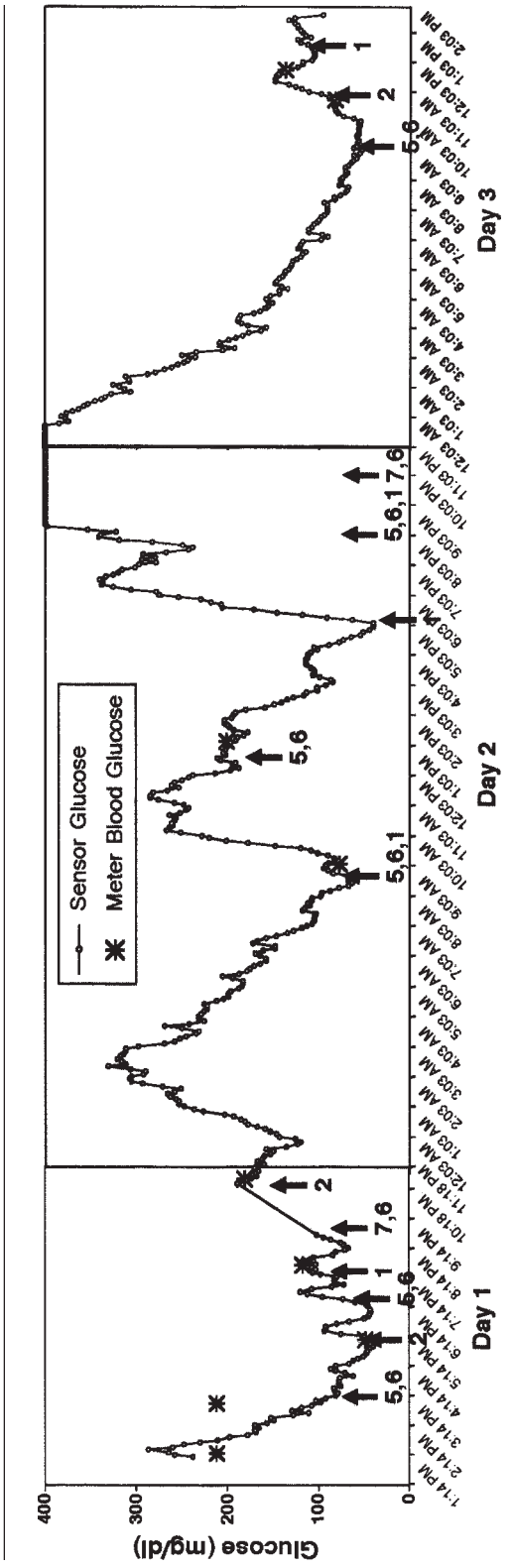

FIGURE 2 Continuous blood glucose monitor (CGMS) pattern in 42-year-old man with type 1 diabetes, treated with regular insulin administered intraperitoneally in each peritoneal dialysis (PD) exchange. The patient's finger-stick blood glucose levels correlated well with the CGMS readings, but did not reflect his long periods of marked hyperglycemia. When no meal was taken, insulin in the PD exchange caused a reduction in blood glucose into the hypoglycemic range. When a PD exchange and food were taken at the same time, the insulin dose was insufficient to prevent marked and sustained hyperglycemia. 1 = meal; 2 = snack; 3 = exercise; 4 = oral agent; 5 = short-acting insulin; 6 = PD exchange; 7 = initiation of cycler exchanges.

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